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The Council have had under consideration the rapid increase of the Society's expenditure on publications. In view of the necessity for economy, authors of papers are urgently requested to see that their communications are put in as concise a form as possible. Delay in decisions regarding publication, as well as subsequent trouble to authors, is often caused by diffuseness or prolixity. MSS. must be type-written or at least written in a legible hand, and properly prepared as copy for press. Type-written transcript should in all cases be carefully revised by the author before being presented. It is desirable that authors should retain copies of their MSS. for reference.

Every paper must be accompanied by a summary not exceeding 300 words in length.

Authors are requested to refer to a Memorandum on Mathematical Notation published in these 'Proceedings,' 1909, Series A, vol. 82, p. 14, and to adhere to the suggestions therein contained, so far as possible.

Authors are further requested to send in all drawings, diagrams or other illustrations in a state suitable for direct photographic reproduction. They should be drawn on a large scale in Indian ink on a smooth white surface, with temporary lettering in pencil. Great care should be exercised in selecting only those that are essential. Where the illustrations are numerous, much time would be saved if the authors would indicate in advance those which, if a reduction of their number is found to be required, might be omitted with least inconvenience.

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The Library and Offices will be closed for cleaning from August 25 to September 6 inclusive. Otherwise they will be open as usual, till 4 o'clock, during August and September.

The Council have directed that the Minutes of the Meetings of the Society shall be sent out as an inset in the 'Proceedings,' separately paged, and shall afterwards be republished in the 'Year-Book.'

The 'Proceedings,' both the Physical and the Biological Series, are sent in the ordinary course by post to every Fellow of the Society who resides within the limits of the Postal Union. On application to Messrs. Harrison and Sons, St. Martin's Lane, London, W.C. 2, these will be bound in volumes, in cloth, for 4s., or the cases for binding may be purchased, price 2s. 3d.

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PROCEEDINGS OF

THE ROYAL SOCIETY.

Section B.—Biological Sciences.

On the Occurrence of Multinucleate Cells in Vegetative Tissues.

By Rudolf Beer, B.Sc., F.L.S., and Agnes Arber, D.Sc., F.L.S., Fellow of Newnham College, Cambridge.

(Communicated by Prof. J. Bretland Farmer, F.R.S.—Received May 23, 1918.)

(Plate 1.)

Historical Introduction.

The typical animal or vegetable cell is now universally held to consist of a protoplasmic body, either naked or enclosed in a cell membrane and containing, as its most essential constituent, a single nucleus. This view as to the uninucleate character of the vegetable cell was first definitely formulated by Nägeli in 1844. A few well-defined exceptions to this rule, such as pollen grains, embryo sacs, etc., were recognised by Nägeli himself, and during the three-quarters of a century which has elapsed since the publication of his work, several other instances of multinucleate cells have been observed by Schmitz, Treub, Johow, Strasburger, Grant, and others. These cases, in which a plurality of nuclei was seen to occur in the cell, were however regarded as isolated exceptions to an otherwise universal rule, and of no general significance.

In 1914 Dr. McLean* observed cells with more that one nucleus in the tissues of certain water plants, while in 1915 Miss Prankerd† published an account of her researches on multinucleate cells. She recorded the occurrence of multinucleate cells in 36 species of plants "widely separated in habit, habitat, and systematic position," including both Vascular Cryptogams and Angiosperms. Miss Prankerd considers, like nearly all of those who have written upon this subject, that the plurality of nuclei arises by amitosis. With regard to the ultimate fate of the nuclei, she does not believe it probable either that all the nuclei but one degenerate in each cell, or that they fuse with one another. She is inclined to suppose that walls may ultimately be formed between the daughter nuclei which have arisen by amitosis.

---

* McLean, R. C., "Amitosis in the Parenchyma of Water-Plants," 'Proc. Camb. Phil. Soc.,' vol. 17, pp. 380–382 (1914), 1 text-fig.

† Prankerd, T. L., "Notes on the Occurrence of Multinucleate Cells," 'Ann. Bot.,' vol. 29, pp. 599–604 (1915), 8 text-figs.

In the same issue of the 'Annals of Botany' as that in which Miss Prankerd's account appeared, we published a preliminary statement of our results upon the same subject.* It is unnecessary to refer to this at length, but we may recall that we recorded a plurality of nuclei in the young parenchymatous tissues of 76 species, chiefly Angiosperms, but including also a Gymnosperm and a Vascular Cryptogam. This phenomenon seemed to us so widespread that we suggested the possibility that a binucleate or multinucleate stage might often intervene as a normal phase of development between the meristematic and adult conditions. The main difference between our results and those of previous writers is that, according to our observations, the plurality of nuclei normally arises by karyokinesis and not by amitosis. Moreover, there are certain peculiarities in the phenomena associated with this mitosis to which we called attention in our note, and which we propose to describe more fully in the present paper.

THE OCCURRENCE OF THE MULTINUCLEATE PHASE.

Binucleate or multinucleate cells have been observed by us in the vegetative tissues of 177 species representing 60 families. They have been found in each of the five classes of living Pteridophyta (*e.g.*, *Equisetum*, Plate 1, fig. 25, and *Lygodium*, Plate 1, fig. 28), and are of very general occurrence in the Gymnosperms (*e.g.*, *Larix*, Plate 1, fig. 23, and *Cryptomeria*, Plate 1, fig. 27), Monocotyledons, and Dicotyledons. The cases in which we have observed them are enumerated in the following list. An asterisk before the name of a species denotes that it is a case in which "phragmospheres" have been observed (see p. 10). All the observations were made on our own preparations, except in the case of those species to which the initials (E.S.) are appended; these have been examined in the late Miss Ethel Sargant's collection of seedling slides.

---

* Beer, R., and Arber, A., "On the Occurrence of Binucleate and Multinucleate Cells in Growing Tissues," 'Ann. Bot.,' vol. 29, pp. 597–8 (1915).

List of Species in which Binucleate or Multinucleate Cells have been observed in Vegetative Tissues.

Family.	Species.	Region in which cells containing more than one nucleus occur.
PTERIDOPHYTA.		
Filicales.		
Polypodiaceæ	_Aspidium filix mas_, L. var. _capitatum_	Prothallus.
	Athyrium filix femina, L. Roth var. _grandiceps_	Prothallus.
	Scolopendrium vulgare, Sm. var. _ramosissimum_	Prothallus.
Schizæaceæ	*_Lygodium japonicum_, Sw.	Mesophyll of climbing frond.
Equisetales.		
Equisetaceæ	*_Equisetum limosum_, L.	Cortex and pith of fertile stem.
	*_E. maximum_, Lmk.	Cortex and pith of fertile stem.
Lycopodiales.		
Selaginellaceæ	*_Selaginella Wildenovii_, Baker	Cortex of stem.
Psilotales.		
Psilotaceæ	*_Psilotum triquetrum_, Sw.	Cortex of stem.
Isoëtales.		
Isoëtaceæ	*Isoëtes, sp.	Lacunar cortex of stem.
GYMNOSPERMEÆ.		
Coniferæ.		
Taxaceæ	*_Taxus baccata_, L.	Pith and leaf bases (including epidermis) of shoot.
Pinaceæ.		
Araucarieæ	_Araucaria Bidwillii_, Hook.	Leaf bases of shoot.
	*_A. excelsa_, R. Br.	Pith of shoot.
Abieteæ	_Larix europæa_, DC.	Leaf bases of shoot.
	Pinus silvestris, L.	Leaf bases and cortex of seedling stem.
Taxodieæ	*_Cryptomeria japonica_, (L.) Don.	Leaf bases of shoot.
ANGIOSPERMEÆ.		
Monocotyledoneæ.		
Pandanaceæ	*_Pandanus Veitchi_, Hort.	Cortex and stelar tissue of root.
Sparganiaceæ	_Sparganium ramosum_, Curt.	Ground tissue of inflorescence axis.
Aponogetonaceæ	_Aponogeton distachyum_, Thunb.†	Lacunar cortex of inflorescence axis; mesophyll of lamina and ground tissue of petiole; mesophyll of spathe.
Alismataceæ	*_Alisma Plantago_, L.	Ground tissue of petiole and inflorescence axis.
Hydrocharitaceæ	*_Elodea canadensis_, Michx.‡	Cortex and epidermis of shoot.
	*_Stratiotes aloides_, L.	Cortex of root; cortex of stolon; mesophyll of leaf.
Gramineæ	_Arrhenatherum avenaceum_, Beauv.	Leaf sheath and ground tissue of stem.
	*_Arundinaria "palmata"_	Ground tissue of stem.
	*_Avena sativa_, L.	Leaf sheath and ground tissue of stem.
	Bambusa metake, Sieb. (= _Arundinaria japonica_, Sieb. et Zucc.)	Ground tissue of stem.
	*_Bambusa "anceps"_	Ground tissue of vegetative stem and cortex of root.

† McLean, R. C. (_l.c._) records the occurrence of binucleate cells in the cortex of _Aponogeton_, sp.

‡ This case was recorded by McLean, R. C. (_l.c._), but phragmospheres were not observed.

List of Species—(*contd.*)

Family.	Species.	Region in which cells containing more than one nucleus occur.
Angiospermeæ.		
Monocotyledoneæ.		
Gramineæ	*Coix Lacryma-Jobi*, L.	Ground tissue of stem.
	Dactylis glomerata, L.	Leaf sheath.
	Festuca, sp.	Ground tissue of stem and leaf sheath.
	Hordeum vulgare, L.	Leaf sheath.
	Leersia oryzoides, Sw.	Mesophyll of first plumular leaf and cortex of mesocotyl (E.S.).
	Lolium italicum, A. Br.	Mesophyll of first plumular leaf (E.S.).
	**Miscanthus sacchariflorus*, Hack.	Ground tissue of stem.
	Secale cereale, L.	Leaf sheath.
	Sorghum vulgare, Pers.	Cortex of mesocotyl, and mesophyll of coleoptile and first plumular leaf (E.S.).
	Triticum vulgare, Vill.	Leaf sheath.
	**Zea Mays*, L.	Cortex of mesocotyl and mesophyll coleoptile (E.S.); and leaf sheath and ground tissue of stem; plerome, periblem and piliferous layer of root.
Cyperaceæ	**Cyperus Papyrus*, L.	Ground tissue of young axis and leaf bases.
Araceæ	**Anthurium Martianum*, C. Koch et Kolb	Cortex of aerial root.
	**A. scandens*, Engler (= *A. violaceum*, Schott)	Ground tissue of stem and leaf sheath. Epidermis of leaf sheath.
	**Monstera deliciosa*, Liebm.	Cortex of aerial root.
	**Philodendron latifolium*, C. Koch	Cortex of aerial root.
Commelinaceæ	**Tradescantia virginiana*, L.	Ground tissue of stem.
	**T. fluminensis*, Vell.	Ground tissue of stem.
Liliaceæ	**Allium angulosum*, L.	Cotyledon (E.S.).
	**A. ascalonicum*, L.	Cotyledon (E.S.).
	**A. Cepa*, L.	Cotyledon (E.S.).
	**A. neapolitanum*, Cyr.	Cotyledon (E.S.).
	**A. Porrum*, L.	Cotyledon (E.S.).
	**Aloe striata*, Haw. (*Hanburyana*)	Mesophyll of first plumular leaf (E.S.).
	**A. sp.* (near *Caspari*)	Mesophyll of first plumular leaf (E.S.).
	Anthropodium cirrhatum, R. Br.	Mesophyll of first plumular leaf (E.S.).
	**Asparagus officinalis*, L.	Cortex, ground tissue and pith of vegetative axis; also (rarely) epidermis and xylem parenchyma.
	**Asphodeline liburnica*, Reichb.	Cotyledon, hypocotyl and mesophyll of first leaf (E.S.).
	Asphodelus albus, Willd.	Cotyledon and first plumular leaf (E.S.).
	A. fistulosus, L.	Hypocotyl (E.S.).
	Bloomeria aurea, Kellogg	First plumular leaf (E.S.).
	Brodiæa lactea, S. Wats.	Mesophyll of first plumular leaf (E.S.).
	Bulbine annua, Willd.	Hypocotyl and mesophyll of first plumular leaf (E.S.).
	Chlorogalum pomeridianum, Kunth.	Mesophyll of first plumular leaf (E.S.).
	Colchicum autumnale, L.	Mesophyll of first plumular leaf (E.S.).
	Convallaria majalis, L.	Pith and cortex of inflorescence axis.
	Cordyline australis, Hook. f.	Mesophyll of first plumular leaf (E.S.).
	**Dipcadi serotinum*, Medic.	Cotyledon (E.S.).

List of Species—(*contd.*)

Family.	Species.	Region in which cells containing more than one nucleus occur.
ANGIOSPERMEÆ.		
Monocotyledoneæ.		
Liliaceæ	**Dracæna Draco*, L.	Cotyledon and mesophyll of first plumular leaf (E.S.) and pith of root of mature plant.
	**Eremurus himalaicus*, Baker	Pith, ground tissue and cortex of inflorescence axis, and mesophyll of leaf.
	**E. turkestanicus*, Regel	Mesophyll of first plumular leaf (E.S.).
	Eucomis nana, L'Hérit.	Mesophyll of first plumular leaf (E.S.).
	Galtonia candicans, Decne.	Cotyledon, hypocotyl and mesophyll of first plumular leaf (E.S.).
	**Gasteria disticha*, Haw.	Mesophyll of first plumular leaf (E.S.).
	**Hemerocallis fulva*, L.	Ground tissue of inflorescence axis and mesophyll of leaf.
	Hyacinthus romanus, L.	Cotyledon and cotyledonary node (E.S.)
	**Kniphofia* (garden var.)	Ground tissue of inflorescence axis.
	Muscari comosum, Mill.	Cotyledon (E.S.).
	M. neglectum, Guss.	Cotyledon (E.S.).
	M. racemosum, Mill.	Pith, cortex and starch sheath of inflorescence axis.
	**Nothoscordum fragrans*, Kunth.	Ground tissue of inflorescence axis.
	Ornithogalum exscapum, Tenore	Cotyledon (E.S.).
	**Polygonatum multiflorum*, All.	Ground tissue of inflorescence axis.
	Scilla festalis, Salisb.	Mesophyll of plumular leaf (E.S.).
	**S. hispanica*, Miller	Pith and cortex of inflorescence axis and mesophyll of leaf.
	S. peruviana, L.	Cotyledon, hypocotyl and first plumular leaf (E.S.).
	S. sibirica, Andr.	Cotyledon (E.S.).
	Tulipa (garden var.)	Ground tissue of floral axis.
	Veltheimia viridifolia, Jacq.	Cotyledon and mesophyll of first plumular leaf (E.S.).
	**Yucca aloifolia*, L.	Mesophyll of first plumular leaf (E.S.).
	**Y. arborescens*, Trelease	Mesophyll of first plumular leaf and hypocotyl (E.S.)
	Y. gloriosa, L.	Cotyledon, primary root and mesophyll of first plumular leaf (E.S.).
Amaryllidaceæ	**Agave spicata*, Cav.	Cotyledon, hypocotyl and plumular leaves (E.S.).
	**Bravoa geminiflora*, Lex.	Mesophyll of first plumular leaf (E.S.).
	Doryanthes Palmeri, W. Hill.	Hypocotyl, and mesophyll of first plumular leaf (E.S.).
Dioscoreaceæ	**Tamus communis*, L.	Pith and cortex of axis.
Iridaceæ	*Crocus vernus*, All. (garden var.)	Parenchyma of leaves and perianth of bud.
	Iris Boissieri, Henriq.	Mesophyll of first plumular leaf (E.S.).
	**I. sibirica*, L.	Mesophyll of plumular leaf (E.S.).
	**I. sp.*	Mesophyll of plumular leaf (E.S.).
Zingiberaceæ	**Hedychium*, sp.	Ground tissue of axis, mesophyll of leaf sheath, and cortex of root.
Orchidaceæ	*Calanthe Veitchii* (garden form)	Leaf bases.
	Neobenthamia gracilis, Rolfe	Root cortex.
Dicotyledoneæ		
Betulaceæ	*Corylus Avellana*, L.	Pith and cortex of stem.
Moraceæ	*Morus nigra*, L.†	Pith and cortex of axis.

† This case has already been recorded by Prankerd, T. L. (*l.c.*), but phragmospheres were not noted.

List of Species—(*contd.*)

Family.	Species.	Region in which cells containing more than one nucleus occur.
ANGIOSPERMEÆ.		
Monocotyledoneæ.		
Polygonaceæ	**Polygonum cuspidatum*, Sieb. et Zucc.†	Pith, cortex and epidermis of the stem.
	**P. senegalense*, Meissn.	Pith of stem.
	**P. Weyrichii*, F. Schmidt.........	Pith of inflorescence axis.
	Rheum "*corallinum*"	Pith of inflorescence axis.
	R. Rhaponticum, L.	Petiole.
	**Rumex obtusifolius*, L.	Pith and cortex of stem.
Nymphæaceæ	*Cabomba*, sp...........................	Lacunate cortex of stem and mesophyll of leaf.
	**Victoria regia*, Lindl.	First internode of seedling, petiole of leaf, peduncle and root cortex.
Ranunculaceæ ...	**Aconitum Napellus*, L.	Cortex and pith of inflorescence axis and vegetative axis.
	**Delphinium* (garden var.).........	Pith and cortex of stem.
	**Thalictrum flavum*, L.	Pith of inflorescence axis.
Papaveraceæ	**Chelidonium majus*, L.	Pith of stem.
	**Glaucium flavum*, Crantz	Pith of inflorescence axis.
Cruciferæ	**Brassica oleracea*, L. (" Savoy ")	Pith of elongating stem of "bolting" plant.
	**Lepidium Draba*, L.	Pith and cortex of stem.
	**Sisymbrium Alliaria*, Scop.	Pith and cortex of inflorescence axis.
	**S. officinale*, Scop.	Pith of stem.
Resedaceæ	**Reseda lutea*, L.	Pith of inflorescence axis.
Saxifragaceæ	**Philadelphus coronarius*, L.......	Pith of stem.
	**Ribes rubrum*, L.	Pith and cortex of vegetative axis.
Rosaceæ	**Kerria japonica*, DC.	Pith of inflorescence axis.
	**Pyrus Malus*, L.	Pith and cortex of stem.
	**Rosa* (garden var.)...................	Pith and cortex of stem.
	**Rubus fruticosus*, L.	Pith of stem.
Leguminosæ	**Galega officinalis*, L.	Cortex and pith of stem.
	**Phaseolus multiflorus*, Willd. ...	Pith of stem.
Geraniaceæ.........	**Pelargonium zonale*, L'Hérit. ...	Pith and cortex of inflorescence axis.
	**Tropæolum majus*, L.	Stem, cotyledonary node, petiole of cotyledon, hypocotyl, cortex and pith of root.
Buxaceæ	**Buxus sempervirens*, L............	Cortex and pith of vegetative axis.
Anacardiaceæ......	**Rhus Coriaria*, L.	Pith of inflorescence axis.
Aceraceæ	**Acer campestre*, L.	Pith of vegetative axis.
Hippocastanaceæ	**Æsculus Hippocastanum*, L.‡ ...	Pith and cortex of stem and inflorescence axis.
Vitaceæ	**Vitis inconstans*, Miq. (= *Ampelopsis Veitchii*)	Cortex and pith of vegetative axis.
	V. pterophora, Baker	Cortex and pith of aerial root.
Malvaceæ	**Althæa rosea*, Cav.	Cortex and pith of axis.
Oenotheraceæ......	**Œnothera longiflora*, L.	Cortex and pith of stem.
Hippuridaceæ......	**Hippuris vulgaris*, L.§	Cortex of stem.
Araliaceæ	**Hedera Helix*, L.‖	Pith and cortex of stem.

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† This appears to be the case recorded under the name of *P. Sieboldii*, by Grant, but phragmospheres were not observed by him. See Grant, A. E., "The Multinucleated Condition of the Vegetable Cell," 'Trans. Bot. Soc. Edinb.,' vol. 16, pp. 38–52 (1886), two Plates.

‡ This case was recorded by Prankerd, T. L. (*l.c.*), but phragmospheres were not observed.

§ This case was recorded by McLean, R. C. (*l.c.*), but phragmospheres were not observed.

‖ Binucleate cells were recorded in the petiole of this plant by Prankerd, T. L. (*l.c.*) but phragmospheres were not observed.

List of Species—(*contd.*)

Family.	Species.	Region in which cells containing more than one nucleus occur.
ANGIOSPERMEÆ. *Monocotyledoneæ.*		
Umbelliferæ	**Ægopodium Podagraria*, L.	Pith and cortex of inflorescence axis.
	**Anthriscus sylvestris*, Hoffm.	Pith and cortex of inflorescence axis.
	**Carum Petroselinum*, Benth. et Hook.	Pith and cortex of inflorescence axis.
	**Caucalis daucoides*, L.	Cortex and pith of axis.
	**Heracleum giganteum*, Fisch.	Pith and cortex of inflorescence axis.
	**Œnanthe fistulosa*, L.	Pith of stem and cortex of root.
	**Siler trilobum*, Crantz	Petiole; pith and cortex of inflorescence axis and of stalks of partial umbels.
	**Smyrnium Olusatrum*, L.	Pith and cortex of inflorescence axis.
Ericaceæ	**Arbutus Unedo*, L.	Pith and cortex of stem.
Oleaceæ	**Fraxinus excelsior*, L.†	Pith and cortex of vegetative axis.
	**Syringa vulgaris*, L.†	Pith and cortex of vegetative axis.
Convolvulaceæ	**Calystegia silvatica*, Choisy	Pith and cortex of vegetative axis.
Polemoniaceæ	*Phlox* (garden var.)	Pith of vegetative axis.
Labiatæ	**Stachys sylvatica*, L.	Pith and cortex of stem.
Solanaceæ	**Solanum nigrum*, L.	Pith of stem.
Scrophulariaceæ	**Antirrhinum* (garden var.)	Pith and cortex of vegetative axis.
	**Calceolaria Pavonii*, Benth.	Pith and cortex of stem.
	**Digitalis purpurea*, L.	Pith and cortex of inflorescence axis.
Acanthaceæ	**Acanthus mollis*, L.	Parenchyma of petiole and inflorescence axis.
Plantaginaceæ	**Plantago major*, L.	Pith of inflorescence axis.
Caprifoliaceæ	**Sambucus nigra*, L.	Epidermis, cortex and pith of stem.
	**Viburnum Lantana*, L.	Pith of stem.
	**Symphoricarpos*, sp.	Pith of stem.
Dipsacaceæ	**Dipsacus laciniatus*, L.	Pith of vegetative axis.
Cucurbitaceæ	**Bryonia alba*, L.	Ground tissue of vegetative axis.
	**B. dioica*, Jacq.	Ground tissue between bundles of vegetative axis.
	**Thladiantha dubia*, Naud.	Cortex and ground tissue of vegetative axis.
	**T. Oliveri*, Cogn.	Pith and cortex of vegetative axis.
Compositæ	**Centaurea nigra*, L.	Pith of stem.
	**Chrysanthemum Parthenium*, Bernh.	Cortex and pith of vegetative axis.
	**Crepis taraxacifolia*, Thuill.	Pith of stem.
	**Dahlia* (garden var.)	Cortex and pith of stem.
	**Doronicum* sp. (probably *Pardalianches*, L.)	Cortex and pith of inflorescence axis.
	**Helianthus Nuttallii*, Torr. et Gray	Cortex and pith of inflorescence axis.
	**H. tuberosus*, L.	Cortex and pith of vegetative axis.
	**Solidago stricta*, Ait.	Pith of vegetative axis.
	**Taraxacum officinale*, Weber	Cortex and pith of inflorescence axis.
	**Tragopogon pratensis*, L.	Cortex and pith of inflorescence axis.

† The occurrence of binucleate cells in these species was recorded by Prankerd, T. L. (*l.c.*), but phragmospheres were not observed.

It will be seen from the above list that the multinucleate condition has been found in the most various vegetative organs. It is most frequently and characteristically met with in the stem, but it has also been observed in many roots—subterranean, aerial, and aquatic (*e.g.*, *Stratiotes*, Plate 1, fig. 29). It occurs in many leaf structures, such as the sheathing leaf bases of a number of grasses (*e.g.*, *Zea*, Plate 1, fig. 3, and *Avena*, Plate 1, fig. 18) and in the basal zone of the perianth of *Crocus*. We have frequently met with binucleate cells in the cotyledon, plumular leaf, mesocotyl and hypocotyl of seedlings. The range of tissues concerned is also very wide, including pith, cortex, epidermis and stele of stem, mesophyll of leaf, cortex and stele of root.

In all the cases in which we have observed multinucleate cells, they are characteristic of young tissues which are actively carrying on the processes of life. For example, in the stem the multinucleate cells first appear in a region just behind the actual meristematic tissue, where preparation is being made for the growth in length of the organ. In *Helianthus Nuttallii* and *Syringa vulgaris* binucleate cells make their first appearance 0·1 mm. behind the stem apex.

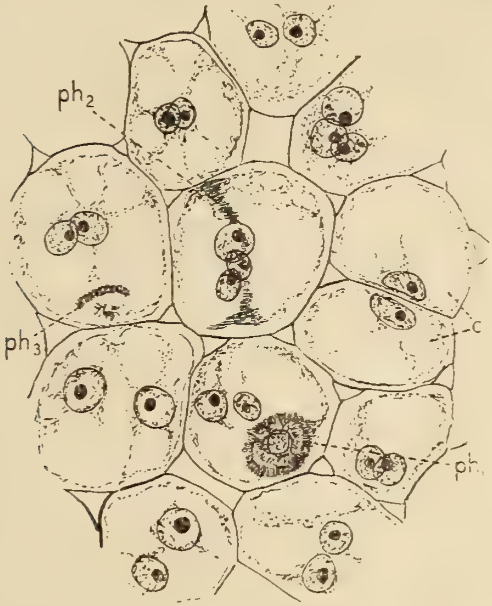
In those stems which have active tissue, capable of carrying out processes of growth, situated at the nodes (such as the stems of Gramineæ and of *Tradescantia*) the multinucleate cells occur predominantly at these spots.

The existence of the multinucleate phase is very easily demonstrated. If for instance, sections be made across the "heads" of *Asparagus*, at the stage at which they are cut for market, preparations such as those shown in text-figs. 1 and 2 will be invariably obtained.

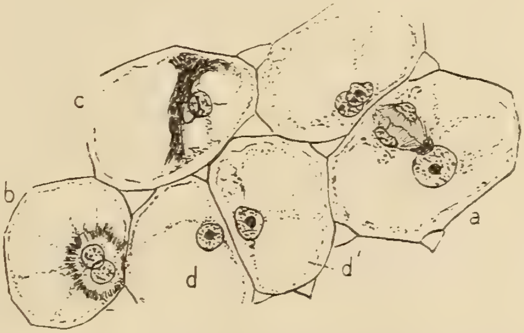
The number of nuclei present in a multinucleate cell varies greatly in different species. Most frequently the cells are binucleate, but in many cases three, four, or even more nuclei may occur in a cell. In the tissues just above a node in the stem of *Zea Mays* (Plate 1, fig. 4) as many as 12 nuclei may sometimes be counted in a single cell, whilst in the young inflorescence axis of *Anthriscus sylvestris*, cells containing 8, 9, and 10 nuclei are not uncommon (*e.g.*, Plate 1, fig. 13).

THE ORIGIN OF THE MULTINUCLEATE PHASE.

Nearly all previous writers have been agreed in holding the view that the origin of the multinucleate condition in a cell is due to the amitotic division of the originally single nucleus. Johow, Strasburger, Grant, McLean and Prankerd have all expressed this opinion; Smolák and Němec are almost alone in having observed a definite instance in which plurality of nuclei became established in the cell by the karyokinetic division of the primary nucleus. They found this to be the case in the plerome cells



TEXT-FIG. 1.—*Asparagus officinalis*, L. Cells of ground tissue between the bundles from a transverse section across the "head" of a shoot gathered on May 6, 1916. (× 535 *circa.*) One phragmosphere (ph_1) is seen at an early stage of development in a cell containing two resting nuclei; a second (ph_2) is at a more advanced stage of development in a cell containing one resting nucleus; a third (ph_3) is only seen in fragmentary tangential section. Two cells (*c*) are uninucleate; these are obviously young cells which have recently divided from one another.



TEXT-FIG. 2.—*Asparagus officinalis*, L. Cells of ground tissue between the bundles from a transverse section 3–4 mm. below the base of the "head" of the same shoot as that from which the section represented in Text-fig. 1 was taken. (× 535 *circa.*) Progressive wall formation is going on in cell *a*, simultaneously with phragmosphere formation in cells *b* and *c*. Just as in Text-fig. 1, the only uninucleate cells are a pair which have been recently formed (*d* and *d'*).

and vessel rudiments of the root of several species of *Euphorbia* and *Ricinus*, but these plants were regarded as exceptions to the general rule according to which multinucleate cells arise by amitotic divisions of the cell nucleus.

We have gone very carefully into the question of the origin of the plurality of nuclei in the multinucleate cells and in more than 100 species we have been able to satisfy ourselves beyond doubt that the several nuclei have arisen by mitotic division. No single instance of direct nuclear division has ever been observed by us in these young active tissues.

THE FORMATION OF PHRAGMOSPHERES.

Certain peculiarities are found in the course of these mitotic divisions which appear to be characteristic of multinucleate cells. We briefly referred to these peculiarities in our preliminary note, when we described them as giving rise to an appearance of a cell lying within another cell. We have now observed this modified form of mitosis in the young vegetative tissues of some 120 species belonging to more than 50 families, including representatives of Vascular Cryptogams, Gymnosperms, and Angiosperms. We therefore feel that we are justified in regarding the process observed as being possibly almost universal in these young tissues.

In cells which are about to become binucleate, the mitosis proceeds normally up to the spindle stage and the cell plate makes its appearance as usual; it does not, however, give rise to any cell membrane. It is apparently resorbed, and the whole phragmoplast* with the associated cytoplasm becomes transformed into a hollow sphere which encloses the daughter nuclei; this sphere gradually increases in diameter, ultimately becoming co-extensive with the cytoplasm lining the cell wall. For this hollow shell, which represents the later development of the phragmoplast, and which, in the case of binucleate cells, replaces the normal apparatus of wall formation, we propose the term "phragmosphere."† The spherical shape is not fully exhibited unless the karyokinetic figure occurs in the centre of the cell. If, as often happens, it occupies a position close to a wall, the sphere at an early stage of its expansion fuses on one side with the primordial utricle and thus loses its symmetry. As the phragmosphere extends, the paired nuclei gradually move apart from one another, while they develop in normal fashion and ultimately assume the ordinary characters of resting nuclei.

The young inflorescence axis of *Anthriscus sylvestris* has furnished some of the most characteristic examples of phragmospheres for critical examination (see Plate 1, figs. 7–12). In many cases only a single phragmosphere occurs in a cell in association with a single dividing nucleus; in others two phragmospheres with both pairs of nuclei at the same stage of division may be observed in a single cell, or one phragmosphere with its nuclei in telophase, a second nucleus in the anaphase of division, and a third nucleus in the resting condition may all occur together in one cell. The history of the phragmosphere in *Anthriscus* is precisely as outlined in the preceding paragraph. By the disintegration of the spindle and the disappearance of the cell plate before it has split into two, the two daughter nuclei, in telophase, are left surrounded by a mass of cytoplasm which is partly kinoplasmic in origin. After a time this cytoplasmic aggregate becomes slightly vacuolar in its interior; the vacuole gradually enlarges, pushing a hollow shell of dense cytoplasm more and more nearly towards the outer boundary of the cell until it merges into the peripheral protoplasm. The spindle fibres break down very completely in *Anthriscus* and the phragmospheres show very little of that appearance of being composed of radiating fibres which is more clearly seen in some of the other plants which we have examined (*e.g.*, *Zea Mays*, Plate 1, figs. 1 and 2). In *Anthriscus* the phragmosphere has a densely granular constitution.

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* See Errera, L., "Ueber Zellformen und Seifenblasen" (Versamml. Deutsch. Naturfor. u. Aerzte in Wiesbaden), 'Bot. Centralbl.,' vol. 34, pp. 395–398 (1888).

† Φραγμός = hedge, fence, etc.; σφαῖρα = sphere.

No previous writer appears to have recognised the existence of phragmospheres, or of anything like them, in ordinary vegetative tissues, but more than one observer has recorded the appearance, within the endosperm, of structures which may possibly be either related to phragmospheres or identical with them. Němec,* for instance, describes in the endosperm of *Secale cereale,* certain bodies, which he calls "Kerntaschen," which bear some resemblance to phragmospheres, but he did not follow out their history in detail. Again, certain figures of endosperm cells, taken by the observers to suggest end views of nuclear spindles, may possibly indicate phragmospheres which have been drawn without a full understanding of their nature.† Among Gymnosperms, Lawson's‡ account of the formation of binucleate cells in the endosperm of *Cryptomeria japonica* suggests the occurrence of structures somewhat resembling phragmospheres. Beyond these few notes, whose relevance is doubtful, we have found nothing in the literature which could possibly be construed as a reference to phragmospheres.

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* Němec, B., 'Das Problem der Befruchtungsvorgänge,' Berlin, pp. 112, 113 (1910).

† Němec, B., *ibid.*, Plate 2, fig. 50; Buscalioni, L., 'Annuario del R. Istituto Bot. di Roma,' vol. 7 (1898), Pl. 17, fig. 77B and Pl. 20, fig. 140.

‡ Lawson, A. A., 'Ann. Bot.,' vol. 18, p. 427 (1904), Plate 29, figs. 28–32.

The Fate of the Nuclei.

The fate of the nuclei in multinucleate cells varies in the different species. In some cases, the plurality of nuclei may persist to a late stage. Thus in the stem of *Syringa vulgaris* binucleate cells still occur in the perimedullary zone as far as 93 cm. from the apex, whilst in *Rosa* binucleate cells were still seen in the cortex of a two-year old stem (Plate 1, fig. 17).

In other cases one or more of the nuclei may undergo degeneration (*e.g.*, *Zea Mays*, Plate 1, fig. 6). This can be well seen in the flowering axis of *Hemerocallis fulva*. In material gathered on May 21 and 24 (1916) the region of the axis just below the base of the young inflorescence showed numerous cases of cells in which one nucleus was normal, while the second was obviously degenerating. The degenerating nuclei are contracted and irregular in form; they seem to be structureless, since they stain more or less homogeneously, while the normal nuclei accompanying them are granular in appearance with a number of nucleoli. Further down the axis the cells are mostly uninucleate.

Although we have spent much time on the matter, no instances of nuclear fusions, as factors in the reduction of the number of the nuclei, have been observed. Neither has any evidence been forthcoming of the existence of a belated cell-division leading to the separation of the nuclei of a multinucleate cell by the interpolation of new cell walls between them. Lobed nuclei have been observed in several instances, *e.g.*, *Helminthostachys*, Plate 1, fig. 24, but a careful and critical examination of these cases has convinced us that these have neither the significance of nuclear fusions nor of amitotic divisions. They merely represent a change of form which is the expression of metabolic activities taking place in the cell. *Statiotes aloides*,* *Asparagus officinalis*, and *Tradescantia virginiana* furnish instances of such lobed nuclei.

The case of *Tradescantia* is particularly noteworthy, since it has long been regarded as one of the classical instances of nuclear amitosis in the higher plants. We have, however, found unmistakable evidence that the plurality of nuclei in the stems of this plant arises by mitotic division accompanied by the occurrence of characteristic phragmospheres. The lobing of the nuclei is here, as elsewhere, a mere change of form, which rarely if ever bears any relation to their multiplication.

After our work on *Tradescantia* was completed, a recent paper by

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* In a former paper by one of us (Arber, A., 'Proc. Camb. Phil. Soc.,' vol. 17, p. 369 (1914)), the origin of the binucleate phase in the roots of *Stratiotes* was wrongly attributed to amitosis. Further work has shown that the binucleate condition arises by karyokinesis accompanied by phragmosphere formation (Plate 1, fig. 29), and that the lobing is a distinct phenomenon.

Schürhoff came to our notice. This will necessitate a somewhat fuller treatment of this plant than we anticipated, or than could be incorporated in the present account. We propose, therefore, to give here only the foregoing brief reference to our results and to reserve the description of further details, together with a discussion of Schürhoff's work, for a separate communication.

THE SIGNIFICANCE OF THE MULTINUCLEATE PHASE.

In the foregoing pages we have called attention to a long list of species in which multinucleate cells occur, not as a chance phenomenon, but as a normal and definite stage in the development of their parenchymatous tissues. We may add that not a single species of flowering plant has been met with in our work in which, after a careful examination, multinucleate cells have not been found to occur to a greater or less extent. We therefore feel that we are justified in concluding that the occurrence of a multinucleate phase is a normal feature in the growth of the majority of the higher plants. It is a phase which in general succeeds the meristematic activity of the cells, and precedes their period of maximum growth. The phase may endure throughout the whole period of growth, and, indeed, traces of it may be found in quite old tissues, or it may give place to a uninucleate condition at an extremely early stage. From the point of view of the development of the individual cell, the multinucleate phase may be regarded as a stage at which the cytoplasm has either temporarily or permanently lost its ability to divide, whilst this power is still retained by the nucleus. A number of cases are already known, both in zoological and botanical literature, in which the cytoplasm and nucleus of a cell are unequally affected by various agencies which exert an influence upon the course of a mitotic division. Němec's* well-known work dealing with the effect of chloral hydrate and other such substances upon cell division is a familiar example, whilst Demoor's† earlier observations upon the effect of chloroform upon the hairs of *Tradescantia* showed that the streaming movements of the cytoplasm are arrested before the mitotic division of the nucleus is interfered with. Loeb‡ has recorded the interesting fact that, at a certain concentration of sea-water (either with sodium chloride, or preferably with magnesium chloride), the cytoplasmic divisions are arrested in a freshly fertilised egg of a Sea Urchin, while the nuclear divisions continue.

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* Němec, B. 'Das Problem der Befruchtungsvorgänge,' Berlin, 1910.

† Demoor, J., 'Archives de Biologie,' vol. 13, pp. 163–244 (1895).

‡ Loeb, J., 'Archiv f. Entwickelungsmechanik,' vol. 2, pp. 298–300 (1896).

Raciborski[*] found that, when an old culture of *Basidiobolus ranarum* was transferred to a 10 per cent. solution of glycerine and the temperature raised to 30° C., cell-division was inhibited, whilst nuclear division continued.

All these observations refer to the establishment of the multinucleate condition as a pathological phenomenon due to the action of abnormal conditions upon the cell. In the series of cases dealt with in the present paper, the phenomenon is entirely due to normal agencies arising in the ordinary life of the plant. The transition from the meristematic to the adult condition apparently affects the cytoplasm first and the nucleus only at a later period. It is interesting to note, however, that the cytoplasm is still capable of taking a considerable share in the mitosis, since a perfectly normal spindle is differentiated which effects the regular distribution of the daughter chromosomes to the poles. A cell plate even is formed at the equator of the spindle, but, so far as could be seen, this plate never splits, and it is apparently at this point that the cytoplasmic mechanism breaks down.

This independent susceptibility of cytoplasm and nucleus is probably not without its significance in the life of the plant.

As we have already stated, the multinucleate phase reaches its most characteristic expression just previous to the maximum period of growth undergone by the region of the stem, leaf, or root in which it occurs. At such a time metabolic activities must be running high in these cells and it is very probable that the multinucleate condition is in some way directly associated with the elaboration of material required for their growth.

Elsewhere we have abundant evidence that the nucleus plays an important *rôle* in cell metabolism, and a large number of facts have been recorded which tend to show that an active interchange of influences, probably both material and dynamic, takes place between the nucleus and the cytoplasm during the elaboration of materials by the cell. This interchange is undoubtedly facilitated by an increase in the nuclear surface.

A number of cases are known in the animal organism in which cells engaged in intense secretory activity exhibit a greatly enlarged nuclear surface. The silk-glands of various insect larvæ, examined by Meckel,[†] Zaddach,[‡] and Korschelt,[§] are characterised by great secretory activity, and this is associated with a much-lobed and branched nucleus. The glandular cells of the parasitic Copepod *Lernanthropus* possess similarly lobed nuclei. The Malpighian tubes of insects are also lined with cells possessing lobed nuclei.

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[*] Raciborski, M., 'Flora,' vol. 82, pp. 107–132 (1896).

[†] Meckel, H., 'Archiv f. Anat. Phys. u. Wiss. Med.' (Müller), pp. 1–73 (1846).

[‡] Zaddach, G., 'Untersuchungen über die Entwickelung und den Bau der Gliederthiere: I. Die Entwickelung des Phryganideneies,' Berlin, 1854.

[§] Korschelt, E., 'Zoologische Jahrbücher: Abtheilung für Anatomie u. Ontogenie der Thiere,' vol. 4 (Part I, 1889), pp. 1–154 (1891).

The so-called "nurse-cells" of many insect ovaries form another striking illustration of cells engaged in intense metabolic activity, in which the nuclei have enlarged their surface by intricate lobing (Korschelt).* In the secretory cells of the mammary gland investigated by Nissen† the same end is reached by the actual division of the nucleus into two or three daughter nuclei, without an accompanying cell-division at first taking place.

Among plants we also find records in the literature of similar facts. The tapetal cells of many stamens form a good instance of this kind. In certain pollen grains, at the time when their cytoplasm is undergoing a rapid increase in quantity, the tube-nucleus becomes amœboid in form.

In *Utricularia* haustoria are formed at both ends of the embryo sac, while part of the placenta, near the base of the ovule, becomes modified as a nutritive tissue, which is tapped by the micropylar haustorium. In *U. oligosperma* this placental nutritive tissue is immensely developed, and shows lobed nuclei and some cells with two nuclei.‡ Here, again, we find the association of lobed nuclei or of several free nuclei with a tissue in which active metabolic processes are certainly taking place.

Altogether there is a considerable body of facts all pointing to the importance of the establishment of as large an area of contact as possible between nucleus and cytoplasm during periods of marked metabolic activity. This end is attained either by the lobing of a single nucleus or through the development of a multinucleate condition.

In another way the establishment of a plurality of nuclei by repeated mitotic divisions may aid the interchange of materials between nucleus and cytoplasm. At each division the nuclear membrane disappears, and the karyolymph mingles directly with the cytoplasm. By this means, materials elaborated within the nucleus rapidly pass into the cytoplasm and can be utilised in cell metabolism. In this connection we may recall the case of the Ascidian *Cynthia partita*. Here the nuclear sap in the developing egg escapes into the cytoplasm, where it becomes located in a definite region; in the course of development it is confined to certain cells which ultimately form the ectoderm. The ectoderm thus " owes its origin to the nuclear sap."

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* Korschelt, E., 'Zoologische Jahrbücher : Abtheilung für Anatomie u. Ontogenie der Thiere,' vol. 4 (Part I, 1889), pp. 1–154 (1891).

† Nissen, F., 'Archiv f. Mikr. Anat.,' vol. 26, pp. 337–342 (1886).

‡ Merz, M., 'Flora,' vol. 84 (Ergänzungsband), pp. 69–87 (1897).

In *Cerebratulus,* also, the whole quality of the cytoplasm of the developing egg is altered by the escape of nuclear substance into it.*

In several of the plants studied by us, notably in the case of *Tropæolum majus,* nuclear degenerations take place at a very early period whilst mitotic divisions of other nuclei are still actively proceeding. It is possible that such a process of continuous nuclear dissolution, accompanied by repeated nuclear multiplication through the growth and division of the remaining nucleus or nuclei of the cell, may contribute to the cytoplasm material which is of importance in cell metabolism.

It appears probable, therefore, that in some or in all of the ways mentioned above, the multinucleate condition is of direct or indirect value to the plant. The multinucleate state may have arisen, in the first place, merely as a chance incident in the transition from young to adult tissue, owing to the higher susceptibility of the cytoplasm to the altering conditions. But once it made its appearance, it would conceivably afford the organism a distinct advantage in carrying out the chemical processes associated with growth, and might tend to become perpetuated as a definite physiological phase in the history of growing members.

EXPLANATION OF PLATE.

(All drawings were made with the aid of a camera lucida. Leitz's 2·50 mm. oil immersion lens was used with various eye-pieces, except in the case of figs. 27, 28 and 29, in which Zeiss's 2 mm. oil immersion lens and C.0.6 were used. The magnifications given below have been reduced to ½ in reproduction.)

Figs. 1 and 2.—Phragmospheres of *Zea Mays,* L. (× 800.)

Fig. 3.—Multinucleate cells from leaf-sheath of *Zea Mays* seedling, 1 inch high. (× 800.)

Fig. 4.—Multinucleate cell from stem of *Zea Mays,* just above first node. (× 800.)

Fig. 5.—Binucleate rudiment of a vessel from the root of *Zea Mays,* just behind growing point. (× 800.)

Fig. 6.—One degenerating and one functional nucleus in one cell of *Zea Mays*; stem about 5 mm. above the ninth node. (× 2000.)

Fig. 7.—Nucleus in prophase of division and another in resting state in a cell of *Anthriscus sylvestris,* Hoffm. (× 2000.)

Fig. 8.—Cell from stem of *Anthriscus sylvestris* with one phragmosphere, one nucleus in anaphase of division, and one nucleus in resting condition. (× 800.)

Figs. 9, 10, 11 and 12.—Stages in development of phragmosphere of *Anthriscus sylvestris.* (× 2000.)

Fig. 13.—Multinucleate cell from stem of *Anthriscus sylvestris.* (× 800.)

Fig. 14.—One of two nuclei from a cell in the older region of stem of *Anthriscus sylvestris.* (× 2000.)

Fig. 15.—Four nuclei in a cell of second internode of young plant of *Anthriscus sylvestris.* Three nuclei are still active and one is degenerating. (× 2000.)

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* MacBride, E. W., "Pres. Add. to Section D (Zoology)," 'Brit. Ass. Adv. Sci. Rep., 86th Meeting,' Newcastle-on-Tyne, pp. 403–417 (1917 for 1916).

Beer and Arber. *Roy. Soc. Proc., B, vol.* 91, *Plate* 1.

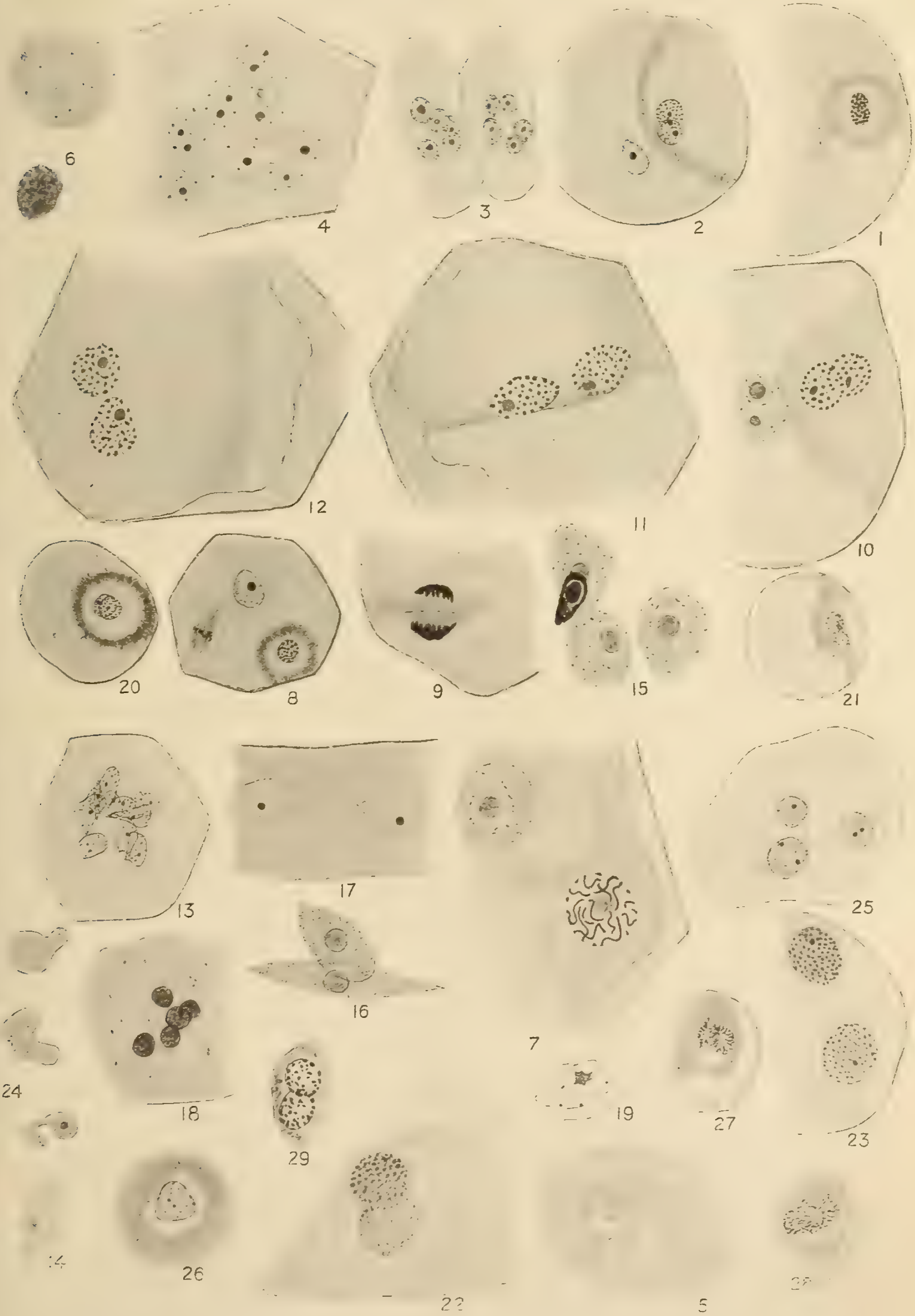
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Fig. 16.—*Rosa* (garden var.). Binucleate cell from young stem. (×2000.)
Fig. 17.—*Rosa* (garden var.). Binucleate cell from stem two years old. (×2000.)
Fig. 18.—*Avena sativa*, L. Parenchyma cell of leaf-sheath. (×800.)
Fig. 19.—*Tropæolum majus*, L. Nucleus from parenchyma of an old stem. (×800.)
Fig. 20.—Phragmosphere from inflorescence axis of *Acanthus mollis*, L., 1 cm. from apex. (×800.)
Fig. 21.—Phragmosphere from stem of *Tropæolum majus*, L. (×800.)
Fig. 22.—Phragmosphere from epidermal cell of leaf base of *Anthurium violaceum*, Schott. (×2000.)
Fig. 23.—Binucleate cell from leaf base of *Larix europæa*, DC (*decidua*). (×2000.)
Fig. 24.—Lobed nuclei from sporangiophore of *Helminthostachys zeylanica*, Hook. (×2000.)
Fig. 25.—Multinucleate cortical cell from sporangiophore of *Equisetum maximum*, Lmk. (×800.)
Fig. 26.—Phragmosphere in a cell from the pith of a stem of *Æsculus Hippocastanum*, L. One of the two daughter nuclei lay exactly beneath the other one, and is not shown in the drawing. (×2000.)
Fig. 27.—*Cryptomeria japonica*, D. Don. Phragmosphere with paired nuclei from a young leaf base. (×1070.)
Fig. 28.—*Lygodium japonicum*, Sw. Cell from mesophyll of climbing petiole, showing two nuclei in a phragmosphere. (×1070.)
Fig. 29.—*Stratiotes aloides*, L. Cell of root cortex from region near apex, showing two nuclei in a phragmosphere. (×1070.)

Concerning Emotive Phenomena.—Part II. *Periodic Variations of Conductance of the Palm of the Human Hand.*

By A. D. Waller, M.D., F.R.S.

(Received June 7, 1918.)

Introductory.

The present communication is in continuation of that made to the Royal Society on November 8 of last year.

At an early stage of the enquiry, I noticed that the emotive effect varied considerably in magnitude upon the same subject (G. de D.) according as she was rested or fatigued, and that it was usually larger during the day than late in the evening; also that the electrical resistance was higher during the evening than during the day.

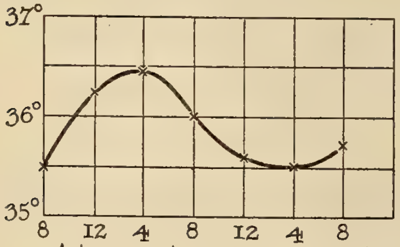
I also noticed on my own hand that the electrical resistance was much higher during the night than during the day, *e.g.*, 200,000 ohms at 2 A.M. as compared with 30,000 ohms at 2 P.M., and that the electrical effects on the palm that accompany natural physiological discharges such as coughing, sneezing, and shouting, as well as the natural responses to a pin prick or burn, were remarkably small. (At such time of depression, however, the electrical effect of yawning continued to be exceptionally distinct.)

Later, when I had come to a definite opinion that the emotive effect is to be regarded as being due to diminished resistance (= increased conductance) rather than to increased electromotive force, I thought it necessary to take observations of the varying resistance of the palm of the hand at all hours of the day and night for periods sufficiently long to bring out the periodicity that appeared to affect the change under the ordinary conditions of daily life.

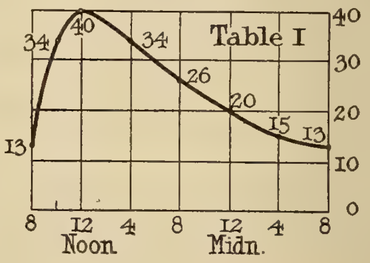
Obviously, an acquaintance with the normal variations of magnitude, apart from its intrinsic interest, is the indispensable preface to an exact study of the emotive response. There is, in point of fact, a general relation between response and resistance that I am not yet able to state with sufficiently complete numerical evidence, but that I hope to deal with on a future occasion.

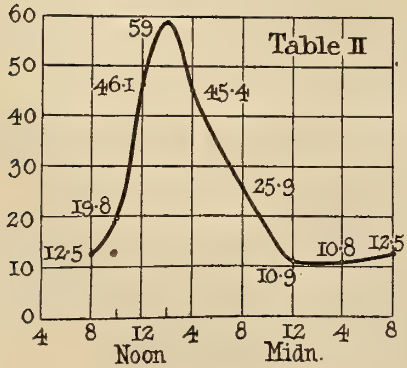
The particular object of this communication is to give the results of observation of the normal course of conductance values during the 24 hours on the palm of my own hand (chiefly the left hand). The series of values is tabulated and plotted. The particular results systematically recorded for the left palm have been occasionally compared with measurements taken on the right palm and on other parts of my own person, as well as upon the palms and other parts of other persons at various times of day.

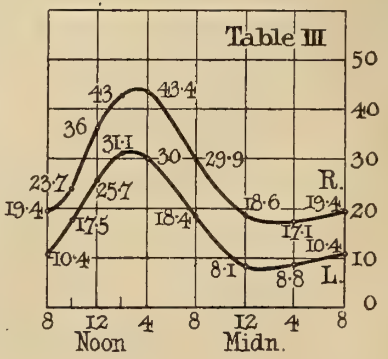
I find it impossible to avoid reference to theoretical considerations in



A temperature curve for the 24-hour cycle







connection with the possible significance of the results. They illustrate in my view the close association between nutrition and what we call "emotion." This theoretical aspect of the facts will be given in a separate paragraph (*infra*, p. 27), where reason is offered for placing the special class of emotive effects in the general category of trophic phenomena rather than in the special class of sudo-motor effects.

Table I.—A.D.W. 2 Leclanché Cells. First Series of Resistance Readings of the Left Hand, led off by electrodes 10 cm.^{2} on Palm and Dorsum.

		γ.	ohms × 1000.
Sunday, March 17—			
8.0 A.M.	No. 391	5	200·00
9.0		19	52·63
9.15		29	34·48
9.30		33	30·30
11.30		88	11·36
1.30 P.M.		44	22·73
2.30		34	29·41
3.0		17	58·82
7.30		25	40·00
8.30		11	90·91
10.30		12	83·33
11.30		20	50·00
Monday, March 18—			
12.30 A.M.		13	76·92
8.0		14	71·43
9.30		20	50·00
10.0		26	38·46
11.0		16	62·50
2.0 P.M.		26	38·46
5.30		35	28·57
7.0		68	14·71
9.0		40	25·00
11.0		30	33·33
12.0		58	17·24
Tuesday, March 19—			
2.0 A.M.		10	100·00
7.0		12	83·33
8.0		32	31·25
9.0		22	45·45
9.30		18	55·56
10.30		20	50·00
2.30 P.M.		44	22·73
7.0		22	45·45
8.30		28	35·71
Wednesday, March 20—			
12.30 A.M.		17	58·82
2.0	No. 396	8	125·00
8.0		12	83·83
8.15		26	38·46
8.30		36	27·78
10.0		50	20·00
5.30 P.M.		36	27·78
8.0		26	38·46
10.30		40	25·00
Thursday, March 21—			
12.30 A.M.		25	40·00
1.30		20	50·00
8.0		16	62·50
9.0		40	25·00
11.0		26	38·46
12.0		40	25·00
2.30 P.M.		38	26·32
7.0		32	31·25
8.0		29	34·48
12.0		35	28·57
Friday, March 22—			
1.0 A.M.		35	28·57
6.0		13·5	74·07
8.0		29	34·48
9.0		60	16·67
11.30		34	29·41
2.0 P.M.		30	33·33
11.30		15	66·67
Saturday, March 23—			
2.0 A.M.		14	71·43
8.35		18	55·56
8.30		22	45·45
8.45		50	16·67
10.15		40	25·00
2.0 P.M.		55	18·18
6.0		40	25·00
11.30		14	71·43
Sunday, March 24—			
8.0 A.M.		22	45·45
9.0		40	25·00
12.0		60	16·67
1.0 P.M.		80	12·50
3.0		40	25·00
7.0		47	21·28
9.0		16	62·50
12.0		24	41·67
Monday, March 25—			
8.0 A.M.		22	45·45
9.0		60	16·67
2.0 P.M.		66	15·15
7.0		50	20·00
8.0		30	33·33

Table II.—A.D.W. Second Series. Hand, Palm and Dorsum.

	Conductance.	Resistance × 1000.
Thursday, March 28—		
10.0 A.M.	20	50
10.15 (shower bath)	50	20
11.30 (breakfast)	53	19
12.0	50	20
2.0 P.M.	55	18
4.30	32	31
8.0	33	30
11.15	11	91
Friday, March 29—		
12.45 A.M.	12	83
5.30	12	83
8.15	11	91
10.0	18	56
10.30 (shower bath)	36	28
11.15	30	33
3.0 P.M.	53	19
6.30	60	17
8.30	33	30
Saturday, March 30—		
1.30 A.M.	26	38
8.0	20	50
9.30 (shower bath)	33	30
9.45	40	25
10.30	40	25
11.30	52	19
4.0 P.M.	70	14
7.0	68	15
8.0 (dinner)	30	33
10.30	20	50
12.0	12	83
Sunday, March 31—		
7.45 A.M.	10	100
8.15	40	25
8·45	23	43
8.50 (shower bath)	60	16
9.15	30	33
9.45	35	28
11.0	30	33
12.5 P.M.	56	17
1.30	90	11
1.35	80	12
3.0	20	50
5.0	60	16
7.0	40	25
8.0	28	35
8.30	20	50
10.0	10	100
Monday, April 1—		
12.0 midnight	8	125
2.0 A.M.	10	100
5.30	13	76
6.30	20	50
7.30	23	43
8.0	30	33
9.30	25	40
10.15	30	33
12.0	34	29
2.30 P.M.	30	33
6.0	30	33
10.0	8	12·5
Tuesday, April 2—		
1.0 A.M.	5	20
8.0	10	100
9.30 (bath)	14	71
10.30	14	71
10.35	20	50
11.0	30	33
11.30	36	27
1.0 P.M.	50	20
3.0	42	23
7.0	38	26
8.0	35	28
9.30 (dinner)	9	11
Wednesday, April 3—		
1.0 A.M.	8	12
8.0	7	14
9.30	16	62
12.0	60	16
3.0 P.M.	40	25
6.0	26	38
10.0	6	16
Thursday, April 4—		
1.30 A.M.	4	25
6.0	5·4	18·5
6.30	13	76
6.40	10	100
6.45	9	11
7.15	14	71
11.0	15	66
11.15 (bath)	30	33
11.30	40	25
12.30 P.M.	15	66
1.0	20	50
3.0	30	33
6.45	88	11
	(after exciting work.)	
7.45 (dinner)	28	35
10.30	9·5	10·53
Friday, April 5—		
8.0 A.M.	10	100
10.0	14	71
12.30 P.M.	70	14
2.30	70	14
6.30	50	20
7.30	22	45
9.30	10	100
11.30	6	16
Saturday, April 6—		
7.30 A.M.	8	12
9.30	6	16
12.0	12	83
3.30 P.M.	22	45
Sunday, April 7—		
7.0 A.M.	12	83
8.30	20	50
9.0	46	21
2.0 P.M.	36	27
8.45	24	41
10.30	10	100

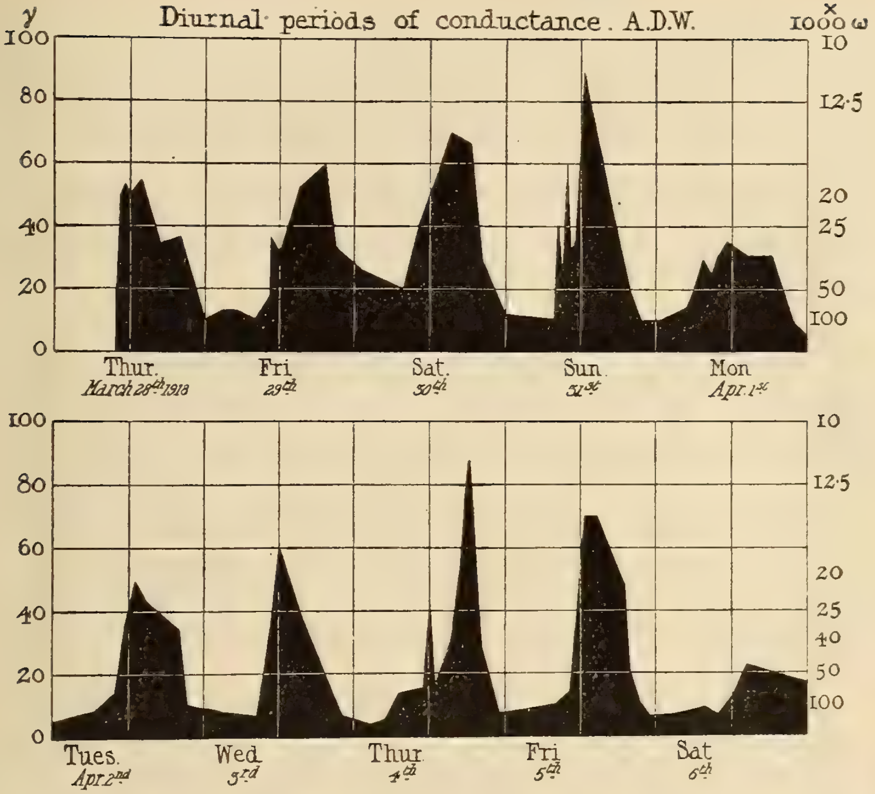


Table III.—April 8 to 21, A.D.W. Third Series. One electrode in Mouth, the other on the Palms of Right and Left Hands.

	L. Conductance.	L. Resistance × 1000.	R.	R.
April 8—				
8.0 A.M.	22	45	36	28
9.30	30	33	42	24
8.0 P.M.	12	83	18	56
April 9—				
3.0 A.M.	6	167	—	—
10.0	8	125	14	71
10.30	14	71	16	62·5
12.30 P.M.	24	42	28	36
11.30	6	167	14	71
April 10—				
10.30 A.M.	8	125	14	71
7.0 P.M.	20	50	32	31
8.0	16	62·5	26	38
11.30	4	250	12	83
April 11—				
8.0 A.M.	10	100	18	56
12.0 midday	30	33	40	25
8.0 P.M.	10	100	26	38
12.0 midnight	6	167	16	62·5

Table III—(*contd.*)

	L. Conductance.	L. Resistance × 1000.	R.	R.
April 12—				
8.30 A.M.	8	125	18	56
6.30 P.M.	55	18	100	10
11.30	12	83	25	40
April 13—				
9.30 A.M.	18	56	25	40
11.30	28	36	40	25
2.0 P.M.	50	20	60	16·7
7.0	18	56	38	26
10.30	8	125	12	83
April 14—				
12.30 A.M.	6	167	8	125
8.0	8	125	14	71
9.0	20	50	26	38
12.0 midday	26	38	30	33
2.0 P.M.	30	33	40	25
6.0	40	25	40	25
7.0	60	16·7	100	10
8.0	26	38	30	33
11.30	5	200	16	62·5
April 15—				
8.0 A.M.	10	100	20	50
10.0	24	41	28	36
5.30 P.M.	50	18	76	13
7.0	28	35	40	25
April 16—				
1.30 A.M.	14	71	24	41
9.30	15	66	25	40
2.0 P.M.	50	18	60	16·7
10.0	7	142	17	58
April 17—				
1.30 A.M.	5	200	15	66
9.0	10	100	20	60
1.0 P.M.	32	31	36	27
6.0	28	36	37	27
8.0	10	100	16	62·5
12.0 midnight	9	111	16	62·5
April 18—				
9.0 A.M.	8	125	14	71
12.0	22	45	25	40
8.0 P.M.	22	45	32	31
9.0	18	56	24	41
12.0 midnight	6	167	8	125
April 19—				
9.0 A.M.	12	83	24	41
11.30	35	28	35	28
4.30 P.M.	55	18	70	14
6.30	55	18	70	14
12.0 midnight	8	125	17	58
April 20—				
9.0 A.M.	11	90	18	56
4.0 P.M.	70	18	80	12
7.0	23	43	35	28

The diurnal periodicity apparent in these Tables is brought out most strikingly by converting them into average values at definite times of day and night. When plotted, these average values give smoothed curves of the normal rise and fall of conductance during the 24 hours. These curves have a course that runs approximately parallel with a normal temperature chart, and are therefore to be regarded as an indication of waxing and waning physiological activity. (*Vide* Charts on pp. 18 and 21.)

Nature of the Emotive Deflection; its Measurement in Terms of E.M.F. and of Resistance (Conductance).

The first question that presents itself in regard to an emotive deflection is whether the increase of current is due to increased electromotive force or to diminished resistance.

I have come to the conclusion that it is due to diminished resistance (*i.e.*, increased conductance), for the following reasons:—

1. An emotive deflection of high apparent voltage (0·5 volt and above), as shown by a galvanometer, is invisible on a capillary electrometer (sensitive to 0·0001 volt).

2. During a persistent emotive deflection of high voltage, a superadded deflection, caused by throwing in an additional calibrating deflection, is augmented in proportion with the augmented conductivity, *e.g.*, during a persistent emotive deflection indicating a great diminution of apparent resistance to 20,000 ohms from an original 40,000 ohms (*i.e.*, conductance × 2), the deflection caused by a superadded calibrating voltage is doubled.

The emotive change of balance shown by a subject placed in the 4th arm of a Wheatstone bridge can be directly measured and expressed in ohms. Or it can be measured and expressed in voltage by balancing against a potentiometer E.M.F.

Or the emotive deflection can be directly measured and expressed in terms of voltage, by means of a calibrating deflection caused by known voltage.

I find it convenient to use a known fraction of a volt for the purpose of calibration, but to express results of experiment in terms of conductance, viz., in reciprocal megohms (γ). The original current is supplied by two Leclanché cells = 2·8 volts. A calibrating deflection by 0·2 and by 0·02 is suitable for low-power and high-power observations respectively, *e.g.*, the palm of the hand of a given subject in the 4th arm of a bridge is found to have a resistance = 20,000 ohms, *i.e.*, a conductance = 50 γ. An emotive deflection on the record is measured to be 25 mm. The calibrating deflection by

0·2 L is found to be 5 mm. The electromotive value of the emotive response is then +1 L or 1·4 volts. Its conductance value is 25 γ by direct reading of the deflection or a rise of conductance from 50 to 75 γ (or reciprocated into terms of resistance = a fall of resistance from 20,000 to 13,333 ohms, *i.e.*, a fall of 6667 ohms).

This fall of resistance can of course be tested by the balancing resistance, but only approximately, because it is a diminishing diminution; in the present example it would be found between 6500 and 6000 ohms. Therefore it is preferable to take out directly the conductance values of deflections with a properly calibrated instrument.

§ 2. *Method.*—By means of a recording galvanometer set up in my dressing room, I took photographic records for short periods (as a rule two minutes, sometimes longer) of the conductance of my left palm (sometimes also of other parts) at all hours of the day and night as happened to be convenient, and plotted the results during a series of days. Records were also taken when possible of other parts, such as the forearm, leg, and foot, for comparison with the palm or skin, which, as stated in my previous communication, is the principal instrument of emotive response.

The hand, galvanometer (suitably shunted), and two Leclanché cells were put up in series, the galvanometer being adjusted by shunt so that a calibrating deflection through a resistance of 20,000 ohms (= a conductance of 50 γ) measured on the photographic plate as nearly as possible 50 mm. (*i.e.*, 1 γ = 1 mm. multiples and submultiples of this adjustment were taken when required).

In the first two series of observations (Tables I and II) the electrodes (zinc discs 10 $cm.^2$, covered by chamois leather moistened with 0·6 per cent. NaCl solution) were fixed to the palm and back of the hand by elastic bands. For the third series (Table III) one electrode was slipped behind the closed lips against the tip of the tongue, and the other fixed to the palm as before, or used as an exploring electrode for other parts against which it was firmly held at the end of an insulating handle by the free hand.

This second or unipolar plan was adopted not only as a simplification for the summary comparison of different parts, but also in order to diminish the risk of surface conduction between adjacent electrodes. I considered that an electrode in the mouth would be equivalent to an electrode applied to the internal surface of the skin, and be of negligible resistance as compared with the resistance of a second area of skin. In justification of this presumption, I measured the resistance offered by:

	ω.	γ.
1. The electrodes applied face to face	100 to 200	10,000 to 5000
2. Electrodes in the mouth and rectum	500 (400 to 600)	2000 (2500 to 1667)
3. One electrode in the mouth, the other on the palm.	20,000 (10,000 to 200,000)	50 (100 to 5)

With one electrode in the mouth, and the other used to explore, observations of differences or changes of conductance are practically limited to the area explored; and it is remarkable how steady the current remains with steadily applied pressure when the electrode is held against the part by an insulating handle, and how much the pressure can be voluntarily varied without causing the current to vary. The mechanical change by which spurious alterations of conductance are most liable to occur, is friction, however slight. A twist of the exploring electrode gives large effects, and it is easy in this way by graduated torsion for a superficial experimenter to imitate the pattern of many kinds of physiological records. Torsion applied at regular intervals is scarcely distinguishable from the staircase summation of effects caused by stimulation of muscle or nerve. Similar photographic records have been made by the friction and torsion of vegetable stems and metallic rods, and given as proof of the life of plants and metals.

In the ordinary course a single galvanometer and recorder are used, and comparisons of different regions of skin are made by successive trials with the same apparatus. For certain purposes such as, *e.g.*, observations of the comparative magnitude of emotive responses at different points to the same excitation, and observations of the gradual changes of conductance occurring in different parts, two galvanometers are used, giving two simultaneous records on the same photographic plate.

The resistance of the skin can be measured in the usual way by Wheatstone's bridge, and for certain purposes this method is more convenient than the arrangement in series. Both methods necessarily include as the chief factor of resistance the polarisation of the skin, which, in my view, is precisely the factor that alters under the influence of emotive effects. I take this measurement by a constant current in preference to that of the strict ohmic resistance obtained by means of the Kohlrausch method with alternating currents, because polarisation is the substantial condition under which the emotive effects are manifested. The ingenious and elegant experiment of Gildemeister, quoted in my first communication, appears to me as having fully established the fact that emotive excitement causes no alteration of resistance apart from polarisation.

An emotive disturbance of balance shown by the galvanometer on the bridge, might be due to a diminution of the electromotive force of polarisation, or to a diminution of the resistance offered by the septum at which polarisation is effected; in either of these cases the balance is restored by a diminution of the balancing resistance and the value of the diminution is read in ohms. If a potentiometer is put in series with the subject in the 4th arm of the bridge as shown by the diagram we shall obtain indications that will enable us to decide between the two alternatives—electromotive force increased or resistance decreased. If the former a known E.M.F. from the potentiometer should give the same deflection before and during an emotive change. If the latter the potentiometer deflection should be increased during the emotive change. And in point of fact this second alternative is what actually occurs; whenever I have measured the resistance in ohms before and during emotive excitement, I have found that the potentiometer deflection was inversely proportional with the resistance. The following, *e.g.*, were the scale readings of a particular observation:—

	Balance.	Potentiometer deflection.
	ohms.	mm.
Before the emotion effect	20,000	20
During the emotion effect	18,000	22

The Question of Sweat Nerves.

Differences of resistance of different parts of the skin as well as alterations of resistance of a given part, are most easily accounted for by attributing them to differences of perspiration. No doubt this simple explanation is sufficient to account for many of the facts of the case, but it does not meet the whole case. The skin of the palm of the hand is abundantly provided with sweat glands, and perspires freely, but it has a higher and more variable resistance than that of the skin of any other part of the body. The skin of the forearm has relatively few sweat glands, and its resistance is lower than that of the skin of the palm. Atropine which arrests perspiration by paralysing the sudo-motor nerves has, in my experience, no effect whatever upon the emotive response. Arrest of the circulation of the limb by an Esmarch band does not influence the emotive response. Immersion of the hand in hot and cold water do not appreciably affect it. We are forced, therefore, to look for a more comprehensive explanation than that of sudo-motor variation.

Admitting, as proved, that the emotive effect consists in a diminution of resistance, we may take the further step of supposing that the diminished resistance is caused by the expansion of ultra-microscopic pores in the membrane between living element and internal medium, and that polarisation at this membrane is diminished. Or quite simply we may imagine that the expanded pores permit of an increased passage of ions. On this view, while recognising that a certain association subsists between emotive and secretory events, we are to regard these events when occurring together as the conjoint results of a common cause rather than as successive results of each other; they are to be regarded as occurring in parallel rather than in series, conjointly sometimes with other evident manifestations of increased physiological activity.

Outflow of water (and discharge of carbonic acid and liberation of mechanical motion) are final events of metabolism. But events in the course of metabolism short of its final consummation give rise to nutritional or trophic changes which have their electrical aspect. Emotive phenomena in my view belong to such previous and deeper changes, although they may also be manifested in company with the final and obvious events of metabolism in company with muscular movements or with sweat or with flushing, all of which are seen as concomitants of sufficiently intense emotion. The ordinary visible signs of emotion—muscular (inclusive of vaso-motor, cardiac and intestinal), secretory (inclusive of sweat flow and salivary secretion)—are more or less under voluntary control and can to some extent be inhibited. The emotive effects manifested electrically by the palm of the hand are not under voluntary control and cannot be inhibited. Suppressed emotions are especially effective in respect of the palmar electrical sign, and this fact constitutes the chief practical value of the sign.

The more perfectly an examinee can control the visible signs of his emotion, the more violently is the galvanometer deflected through the palm of his hand by reason of his suppressed emotion.

The emotive phenomenon belongs to the category of trophic changes; it is a sudden brief intensification of a slowly fluctuating state of nutrition.

I have become accustomed to designate the brief change alone as "emotive," and to refer to the prolonged change alone as "trophic." I imagine the changes as brought about through what we are accustomed to designate as "trophic" nerve fibres, and find no necessity for invoking the existence of special emotive as distinct from general trophic channels, nor for assigning emotive effects to sudo-motor fibres exclusively, any more than to vaso-motor fibres, or musculo-motor fibres. The emotive effects are, in my view, essentially trophic ; in its fullest and most intense forms emotive discharge from the central nervous system may occupy any centrifugal nerve to any organ of the body.

Emotion may be manifested by a flow of tears, or an outbreak of sweat, by a frown or gesture, or verbal ejaculation ; if at such time we had the palm of the excited person connected with the galvanometer we should see an emotive deflection, but the latter is not due to the tears or sweat any more than to the muscular movement, and it may be most marked when all these ordinary signs of emotion are absent.

While it is, no doubt, true, that the deflection is due to diminished resistance, and that perspiration causes diminished resistance, it does not follow, logically, that the deflection is due to perspiration, since there are many occasions on which the emotive fall of resistance occurs without augmentation of either sensible or insensible perspiration.

I have utilised in justification of this last statement a test that I devised some years ago for the measurement of insensible perspiration.*

A capsule containing a deposit of calcium chloride is left inverted against the palm of the hand for a period of, say, 10 minutes, and shows by its gain of weight the amount of water that has been "insensibly" exhaled and evaporated from the area covered by the capsule. Thus, *e.g.*, the insensible perspiration in a room, with a temperature of 20°, was found to be for a period of 10 minutes from an area of 20 cm.^2 : palm of the hand, 24 mgrm. H_2O ; flexor surface of the forearm, 5 mgrm. H_2O, *i.e.*, the greater perspiration was from the skin of higher electrical resistance.

[*Note added, June* 30.—The statements made in this communication were supported by the following experiments carried out during the meeting (June 27).

First Experiment.—Dr. Waller showed, with one electrode, A, on the palm of his left hand, and the other electrode, B, in his mouth, in series with two Leclanché cells and a galvanometer (shunted)—

(*a*) That the conductivity of the palm was at once of a steady maximal value (approximately 50 γ or 20,000 ω).

(*b*) That alterations of contact at B, caused by movements of the tongue in speaking, caused no visible alteration of deflection, signifying that the resistance at B was of negligible value.

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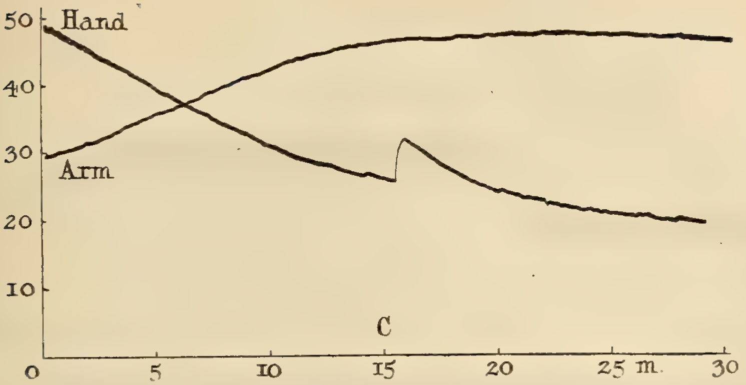
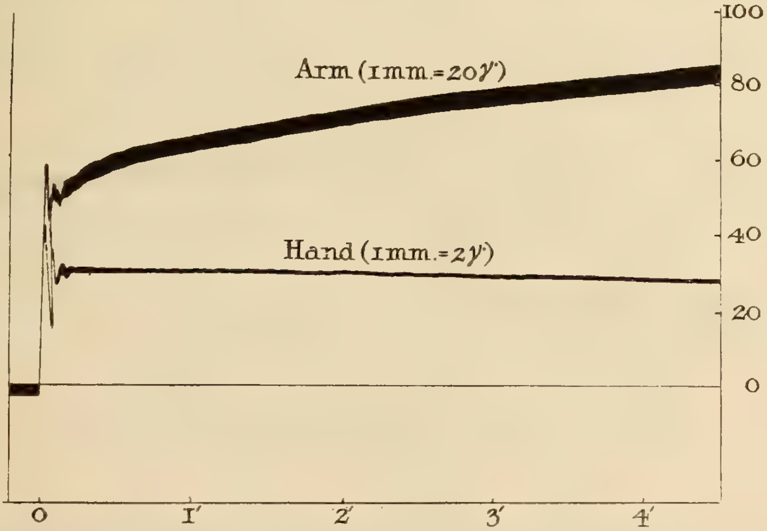
* Waller, 'Proceedings of the Physiological Society,' Nov. 11, 1893 ; 'Journal of Physiology,' vol. 15.



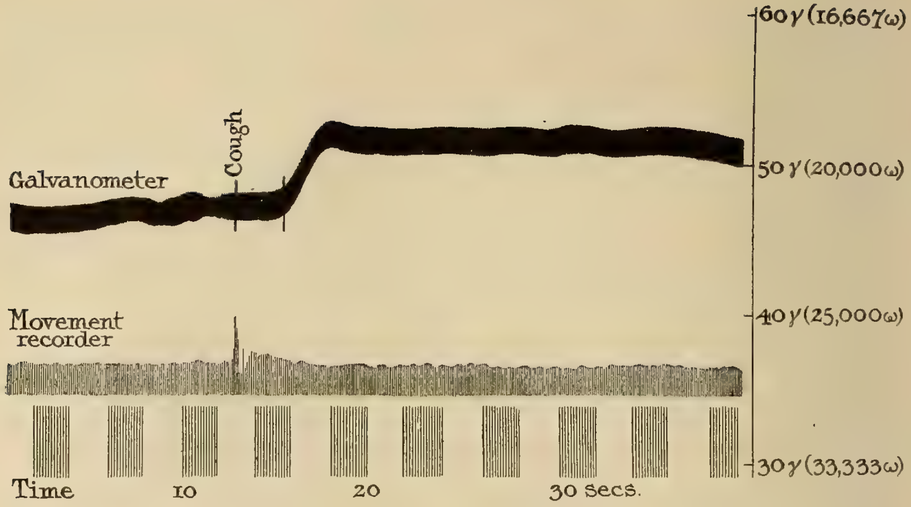
Successive records taken on the same plate by the same galvanometer of the deflection given by a current of two Leclanché cells passing through the hand and through the forearm for 30 minutes. The galvanometer was shunted to 1/10th for the forearm.

		Hand.	Forearm.
Conductance	at beginning	44 γ	260 γ
	at end	17·5	410
Resistance	at beginning	22,730 ω	3846 ω
	at end	57,140	2439

At the time indicated by the vertical bar, C, a voluntary cough was made, which produced no effect upon the forearm, but gave an emotive effect = + 6 γ from 23 to 29, or from 43,480 to 34,480 ω.

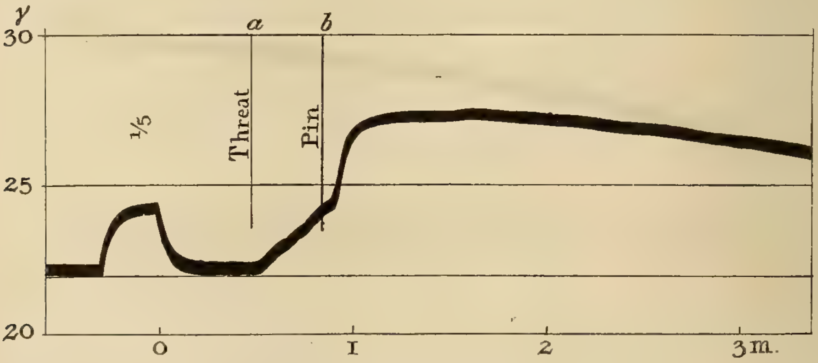


Simultaneous record taken upon two similar galvanometers of the conductance of the hand and of the forearm during the first four minutes of the establishment through each of a current of two Leclanchés. The forearm galvanometer is shown to be 1/10th of the sensitiveness of the hand galvanometer. The current through the forearm is rapidly increasing (= from 135 to 215 γ conductance; or from 7407 to 4651 ω resistance). That through the hand is slightly decreasing (= from 15·75 to 15 γ conductance; or from 63,500 to 66,667 ω resistance).

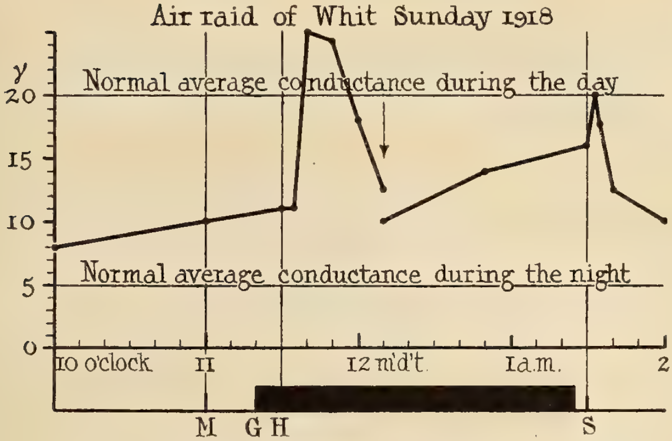


Lost time (2 secs.) of the emotive effect produced in the palm of the hand by a voluntary cough.

The moment of the cough is indicated by an air recorder strapped to the forearm, of such delicacy that it shows the volume-pulse (80 per minute) as well as the mechanical movement of the forearm accompanying the cough. The dimensions of the effect are as follows: lost time = 2 secs.; rise of conductance from 46·5 to 51·25 γ (= fall of resistance from 21,510 to 1950 or 2010 ohms, *i.e.*, in laboratory language, a "2000-ohm cough").



A.M.W.—Normal emotive response, 4 P.M., May 19, to *a*, the threat of a pin-prick; *b*, a real pin-prick.



A.M.W.—Emotive response to the Air-raid of May 19–20.
M. First warning by maroons.
G. Commencement of gunfire, etc.
H. Aeroplane hum very audible.
S. Second warning signal by siren.

(*c*) Electrode A was transferred to the forearm, B being kept in the mouth. The deflection steadily increased to about twice its original value.

Second Experiment.—A pair of electrodes was fixed to the left hand of the President (Sir J. J. Thomson), placed in the 4th arm of a Wheatstone bridge in series, with a potentiometer delivering 1/5 Leclanché for calibration. Main circuit supplied by two Leclanché cells. Balance having been established, Dr. Waller proceeded to stand behind the President's chair, and a deflection of approximately 60 cm. of scale occurred, representing the emotive response of the President to Dr. Waller's presence behind him. The electromotive value of the deflection was approximately 0·5 volt; expressed in terms of a reciprocal megohm, it had a conductivity value of $+6\,\gamma$, *i.e.*, an increase of 20 per cent. of the original conductivity ($30\,\gamma$ or $33{,}000\,\omega$).]

Concerning Emotive Phenomena.—Part III. *The Influence of Drugs upon the Electrical Conductivity of the Palm of the Hand.*

By A. D. Waller, M.D., F.R.S.

(*With a Note on Atropine by* R. Markbreiter.)

(Received February 22, 1919.)

I possess comparatively few data concerning the action of drugs upon "Emotivity" or, to put it more specifically, upon the electrical resistance of the palm of the hand. Except as regards atropine, with which I have made many observations to test the sudo-motor theory of the reaction, I find in my notes only one satisfactory observation upon each of the following drugs: alcohol, chloroform, morphia, which I will transcribe. Obviously, a single observation of any drug can give only a single facet of its action under the particular conditions of experiment. It will, however, be clear that the results have, in each instance, been such as might be anticipated on general principles with one notable exception, viz., atropine.

Experiment 1: *Alcohol.*—A healthy subject, F. G., aged 30, with an initial hand conductance = 17γ (= 60,000 ohms) gave emotive reactions = 3γ to the threat of a burn (match struck) and 2γ to an actual slight burn, immediately before and immediately after the ingestion of 50 c.c. of whisky. The conductance remained unaltered at 17γ.

50 c.c. of whisky or 12½ fl. ʒ is the normal "nightcap" quantity to which the subject is accustomed, and has not produced any marked physiological effect. Experiments with larger amounts of alcohol are required, but are postponed.

Experiment 2: *Chloroform.*—The same subject, F. G., inhaled chloroform at 2 per cent. for one minute, inducing what he estimated subjectively as the first degree of anæsthesia. The conductance of his hand was 20γ (50,000

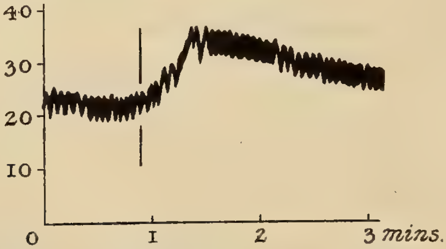


Fig. 1.—Inhalation of chloroform during first half of second minute.

ohms) before and 33γ (30,000 ohms) after the inhalation as is shown by the record (No. 291).

The sudden augmentation of conductance, subsiding gradually to its normal level in 3 to 4 minutes, is due to the excitant action of chloroform. The amount of vapour that was absorbed during the half minute may be estimated as having been approximately 200 c.c. or 1 c.c. liquid or 1·5 grm. This amount according to my previous estimations is physiologically equivalent to 60 grm. of ethyl alcohol which might be contained in say 250 c.c. of whisky. The toxic value of the reagent used has thus been about five times as great in the second experiment as in the first and the results have been in correspondence with this difference.

Experiment 3: *Morphia.*—G. de D., aged 25, received a hypodermic injection of morphia hydrochloride (0·016 grm.) in the course of a prolonged sitting during which she had been subjected to considerable excitement. Her hand conductance had thereby been raised above normal, standing at 160 γ (6,250 ohms) in place of her normal which is 50γ (20,000 ohms) at that time of day —2 to 5 P.M.

The course of the observation was as follows:—

Time.	Conductance.	Resistance.	Remarks.
min.	gemmhos.	ohms.	
0	140	7,100	Observation starts after a considerable excitement.
5	150	6,700	
10	140	7,100	
15	120	8,300	Quieting down.
18	100 to 140	10,000 to 7,100	Morphia injected.
20	140	7,100	
25	130	7,700	
30	140	7,100	
35	140	7,100	
40	80	12,500	Going to sleep.
50	60	16,700	Asleep.
h. m.			
1 0	60	16,700	
1 5	60 to 90	16,700 to 11,100	Awakes with nausea.
1 10	60	16,700	Sleeps again.
1 15	40	25,000	
1 20	50	20,000	
1 25	110	9,100	Wakes with nausea.
1 30	100	9,100	Remains awake with nausea.
1 35	100	9,100	
1 40	40	25,000	Goes to sleep again.
1 45	40	25,000	
1 50	40	25,000	
1 55	80	12,500	Wakes and is sick.
2 0	60	16,700	
2 10	60	16,700	Quiet and gradually feeling better.
2 20	60	16,700	
2 30	50	20,000	

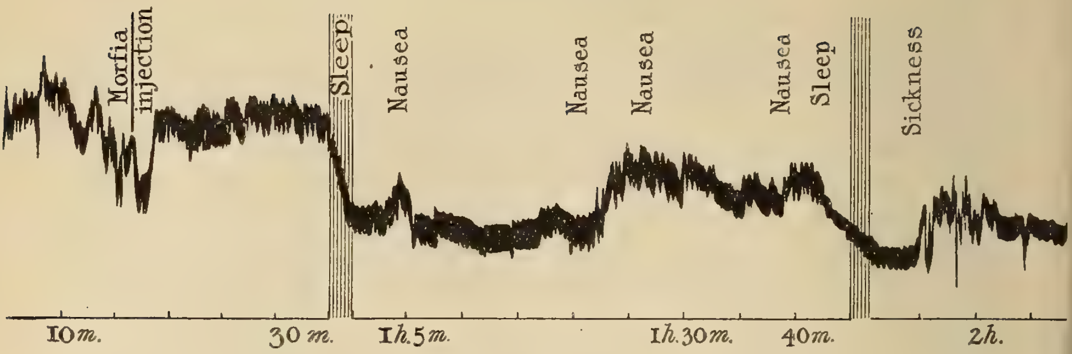


FIG. 2.—Morphia.

Experiment 4: *Atropine.*—As soon as one has realised that the emotive reaction is a true fact, one thinks of the action of sudo-motor nerves, and tests the effect of atropine. I did so at the outset of my experiments, and, being familiar with the sudo-motor effects demonstrable on the cat's foot, as well as with their prompt abolition by atropine, I did so in the expectation of a positive result. I found it quite impossible to persuade myself of the expected result, either by repeated trial and simple observation of the galvanometric spot or by means of photographic records. By simple observation, it is of course possible to arrive at a wrong conclusion; the response varies sufficiently under different conditions for one to think that there has or has not been an abolition or a diminution by atropine. I have therefore taken careful records of the palm treated by local application of belladonna for 48 hours, followed by hypodermic injection of atropine, with the usual general effects, dilatation of pupil, dryness of the mouth, high-pulse frequency, headache and nausea without producing, as far as I could see, the slightest effect upon the emotive response. My observations have not been very numerous—about twenty in all—but the most recent of them have been as thorough as I thought justifiable upon the human subject, and at the conclusion of the last trial I decided not to repeat it. In the face of the statements of other observers, it is useless to assert that atropine has no effect upon the emotive reaction, and I shall content myself with the statement that I have failed to observe it, and that I have recorded instances where it remained, and was apparently of normal magnitude in a subject presenting the usual signs and symptoms of atropine poisoning.

I shall not venture to assert that atropine, in larger dose than I care to employ, like any other powerful toxic agent, such as morphia or chloroform, will not abolish the emotive response. My statement is limited to the specific effect of a small dose.

Atropine Experiment. Plates Nos. 644 to 647. Belladonna plaster on left palm for 48 hours; subcutaneous injection to fore-arm (atropine sulphate, 0·00195 grm.) at end of first half-hour of experiment.

Time.	Conductance.	Remarks.
mins.		
0	50	Start.
5	58 + 1	Sham pin.
7	62 + 2	Real pin.
10	62 + 2	Real burn.
15	68	
20	70 + 3	Pin-prick.
25	70	
27	70 + 3 + 6	Burn.
30	78 + 8	Atropine injection, three tabloids (Wellcome).
35	78 + 2	
40		
45 (No. 746)	80	
52	78 + 2	Pin.
55	74 + 3 + 4	Discomfort.
60	78 + 2	Burn.
63	78 + 2	Spontaneous movement.
65	70 − 8	
70	$\frac{1}{5}$ volt 68 − 4	
75 (No. 747)		
78	74 + 2	Pin.
90	60	
99	60 + 2	Burn.

Pilocarpine, by reason of its sudorific action, might be expected to produce an unmistakable augmentation of conductivity. In two experiments, made *ad hoc*, augmentation of conductivity has been well marked, but the emotive reaction was not appreciably altered.

Experiment 5.—Effect of a Subcutaneous Injection of 0·005 grm. Pilocarpine Hydrochloride.

Time.	Conductivity.	Resistance.
mins.	gemmhos.	ohm.
0	37·5	26,670
10	48	20,830
20	52	19,230
30	62	16,130
40	62	16,130
50	55	18,180
60	51	19,610

The general conductivity of the skin of the hand has been doubled in consequence of the pilocarpine injection. The emotive effects of the four pricks have been + 20, + 12, + 10, and + 5. That of the sensation of nausea was +25. I do not regard the differences as showing any distinct alteration of emotivity under the influence of pilocarpine. The effect on the skin perspiration and upon salivary secretion was evident; 60 c.c. of saliva were secreted during the 70 minutes.

Experiment 6.—Effect of a Subcutaneous Injection of Pilocarpine Hydrochloride 0·006 grm.

Time.	Conductance.	Resistance.	Remarks.
mins.	gemmhos.	ohms.	
0	40	25,000	
10	45	22,000	
12	40 rising to 60	25,000 to 16,700	Pin-prick 1.
18	50 ,, 80	20,000 ,, 12,500	Injection of pilocarpine.
30	80 ,, 92	12,500 ,, 10,900	Pin-prick 2.
35	80 ,, 105	12,500 ,, 9,500	Nausea.
44	70 ,, 80	14,300 ,, 12,500	Pin-prick 3.
50	60	16,700	
58	45 ,, 50	22,000 to 20,000	Pin-prick 4.
72	35 raised to 45	28,500 ,, 22,000	Remoisten electrodes.

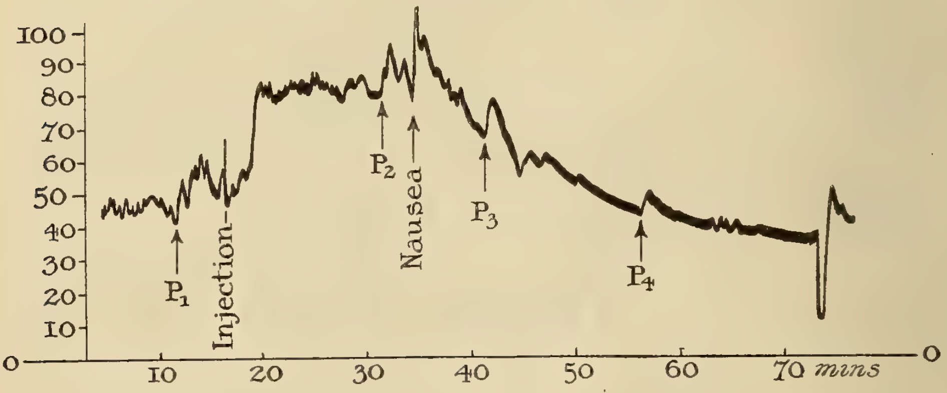


Fig. 3.—Pilocarpine.

The negative result observed after pilocarpine is in agreement with observations I have made in cases of shell-shock where perspiration has been excessive without any corresponding augmentation of conductivity and an emotive reaction greater than normal. The condition is illustrated in the following case:—

Sergeant B......, D.C.M., under the care of Colonel Mott and Captain Golla at the Maudsley Hospital. Shell-shock. Excessive perspiration (electrodes 10 cm.^{2} on back and palm of hand):—

Conductivity	37·5 γ	= 27,000 ohms.
Response to threatened pin-prick	+15 „	= 7,600 „
Real pin-prick	+30 „	= 11,900 „
Threat of a burn	+25 „	= 10,700 „
Real burn	+35 „	= 12,900 „

The significance of this case consists in the fact that during a presumably maximal action of sweat glands, the conductivity of the skin has not exhibited great augmentation, whereas great augmentations of conductivity have been caused by emotive excitement without any visible alteration of the sweat discharges. Expressed in terms of resistance, we have for the sweating hand a resistance—27,000 which has suffered diminutions of 7,600–12,900 ohms, *i.e.*, has fallen to 19,400–14,100 ohms during emotive excitement. The obvious conclusion to be drawn is that the emotive changes have not been caused by augmented activity of the sweat glands.

In these experiments we have to bear in mind that the dread of swallowing medicine or actual pain of a hypodermic injection have emotive effects that must be distinguished from the actual influence, if any, of the drug itself. After the first experiments I found it necessary to avoid the complication of several sorts of emotive stimuli, and to take only one sort, viz., a pin-prick as the regular standard test. For self-observation the most convenient standard test of emotivity consists in a voluntary cough, or a sneeze excited by tickling the nostril.

It is interesting to watch the galvanometer while a patient takes a nasty medicine to order at a given moment. A pronounced emotive deflection occurs while the patient is making up his mind, and the observer soon learns to recognise by the galvanometer whether the patient has swallowed his medicine or has failed to make up his mind.

The deflection is single or double according as the imaginary unpleasantness has, or has not, been followed by the real unpleasantness. The sensation of nausea is always attended with a large augmentation of conductance.

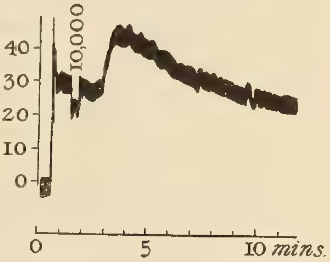


Fig. 4.—Castor oil.

There are several facts pointing to the relative independence of electrical from other emotive manifestations, secretory or muscular. Among such facts, the most cogent would be the failure of its abolition by atropine (if admitted), and its presence in undiminished degree during intense secretory action (*vide supra*, p. 36). Nevertheless, the association between various emotive manifestations is such that it would be hazardous to assert that they are absolutely independent of each other; perhaps the relation between the electrical and the secretory change can be intelligibly conveyed by saying that whereas the electrical is not a consequence of the secretory phenomenon, *i.e.*, in series with it, both phenomena are consequences of metabolic activity, *i.e.*, in parallel with each other. Even this limited degree of association requires the further qualification that our parallel movements may be in opposed directions, *i.e.*, an increased emotive deflection may be accompanied by decreased secretion. Fear dries the mouth, and the following experiment appears to show that emotion can dry the skin, although, as is well known, the usual effect of emotion is increased perspiration. Grief excites tears, but extreme grief may be tearless.

Experiment 7.—Miss G. de D., a Belgian lady, who is familiar with these experiments, and highly skilled in the laboratory methods of carrying them out, prepared apparatus to take a 40-minute photographic record of her own emotive state by means of electrodes on back and palm of one hand and measured the insensible perspiration of the other palm by means of an inverted glass $CaCl_2$ capsule strapped to the hand and giving by its increase of weight a measure of insensible perspiration. She set herself the task of remaining emotionless during the first 20 minutes, and emotionally unhappy during the next 20 minutes. At the end of these two periods the capsule was weighed showing a smaller increment of weight during the emotional than during the emotionless period, and the developed photogram proved conclusively that these states of mind had been successfully maintained. (The psychological interest of this fact is obvious, it implies considerable power of self-control, of which subjects are more or less capable; the emotional state in G. de D. was secured by voluntary recollection of air-

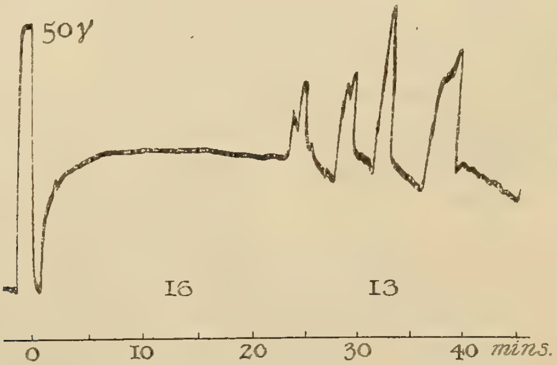


Fig. 5.

raids, and of the "military execution" of a Belgian witnessed at Termonde in 1914.)

The experiment was repeated three times on subsequent days with similar results, viz. :—

Quiet.	Excited.
16 mgrms.	13 mgrms.
10 „	8 „
24 „	15 „

I have taken great pains to satisfy myself about the action of atropine upon the emotive response and have failed to find any evidence that it possesses any action at all upon the response. A negative finding is always unsatisfactory and demands greater bulk of evidence than does a positive finding. But in reality the case is reversed, it is absence of response during atropinisation that is the negative evidence, its presence that is positive evidence. I have repeatedly witnessed emotive response during what I regarded as being adequate if not profound atropinisation. Nevertheless I found it difficult to rest satisfied with the result in presence of the opposite findings of other workers. My statement that such responses do actually occur, and that therefore the emotive effect is not exclusively independent of sweat glands, is based upon observations taken at the outset of the enquiry before its photographic technique had been mastered, and at the last of these earlier observations the result was so clear and the general symptoms of atropinisation so unpleasant that I have not felt it justifiable to repeat the trial for the sake of obtaining a record. In case some future observer may consider it necessary to do so, I shall state what, in my opinion, would constitute satisfactory photographic evidence. A series of emotive responses to a given stimulus, say a pin-prick, then an injection of atropine sufficient to produce general symptoms and abolition of the emotive response.

Local application of atropine by liniment and plaster affords a less convincing test, and can be of value only after the general test has shown atropine abolition. The local test produces no general symptoms, and no local effect. I possess simultaneous records by two galvanometers, one connected with the normal hand the other with the atropinised hand, that exhibit identical large emotive responses in both palms. But as matters stand this is at most a "weak confirmation" of the negative result witnessed in association with general symptoms after subcutaneous injection.

I make no attempt to account for the positive results witnessed by other observers.[*] Gildemeister, in the same issue of the 'Münchener Wochenschrift,' considers that Leva's observations establish with certainty that the p.-g. phenomenon depends upon the sweat glands.

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[*] Veraguth, *loc. cit.*, p. 185 ; Leva, 'Münchener Medic. Wochenschr.,' 28 Oct., 1913, p. 2388 ; Wells and Forbes, in 'Archives of Psychology,' published by the Columbia University (Verbal communication by Dr. Forbes to A. D. W.).

Leva's evidence is particularly strong: he states that in 10 cases the response was abolished by the subcutaneous injection of 1 mgrm. of atropine sulphate,

After 10 to 15 mins. galvanometer deflection, as before,
„ 15 „ 25 „ „ „ distinctly smaller,
„ 30 „ „ „ invisible.

A very graphic description indeed, sufficient to convince any unprejudiced hearer, but nevertheless not in my opinion finally conclusive. Leva is obviously a firm believer in the abolition by atropine, it is possible for a firm believer to watch a more or less steady galvanometer and to *see* responses before atropine and to *not see* responses half an hour later. I had in my laboratory, working at this point for months, a very convinced believer, Mrs. Markbreiter, B.Sc. London, whose report I subjoin. For my own part I do not consider Mrs. M.'s results to be confirmatory of Leva's statements and I regard the latter as inconclusive.

The Effect of Atropine on the Emotive Response.

By Rita Markbreiter, B.Sc. Lond.

Otto Veraguth in 1904 gave the name psycho-galvanic reflex to the following phenomenon, namely, if a current is passed through the body of a subject who is then excited emotionally, either psychically or through one or more of the sense organs, the said current is increased.

Investigators at once asked themselves the question which set of nerves and what cells of the body were concerned in this electrical change. Certain facts pointed to the sweat glands being primarily concerned, and it was thought the emotions aroused caused secretion of the sweat glands to take place, and so gave changed conductivity.

Atropine has the effect of temporarily stopping glandular secretion, and therefore provides an obvious means of proving the above statement.

Veraguth was the first to carry out an atropine experiment: he placed a belladonna plaster on his subject for several days, then took it off, washed the hands with atropine sulphate, and connected the subject with the instrument. He found that the p.-g. reflex was much smaller than in the normal subject, but not entirely extinguished.

Dr. J. Leva in 1913 experimented to find out whether different parts of the body gave different psycho-galvanic results. He found that the field of sweat glands and the field of the p.-g. reflex corresponded with one another in a very close manner. To add a conclusive proof he again carried out atropine experiments. The following is an extract from his paper :—

"Ten subjects, who showed the p.-g. reflex in a normal manner were injected subcutaneously on any spot chosen with 1 mgrm. of atropine sulphate. The subsequent observations were as follows :—Directly after the injection, and for the next 10 to 15 minutes, the galvanometer movement was of normal size. After 15 to 25 minutes it became noticeably smaller, and after about 30 minutes, even after the strongest stimulus, no galvanometer reaction could be noticed."

As neither Veraguth nor Leva publish photographs nor give any very detailed accounts of these experiments, it was thought worth while to carry them out yet once again.

Apparatus Used.

In the first two experiments a single circuit was used, and only the atropinised hand was connected to the instrument—the circuit consisted of two Leclanché cells, a resistance box, the subject, shunt, and galvanometer ; the resistance box and subject being put into the circuit separately or simultaneously by means of keys.

It was thought, however, that no conclusive results could be arrived at unless simultaneous records were taken of an atropinised and a normal hand, therefore the remaining experiments were carried out on Dr. Waller's apparatus, which consisted of two circuits, each of which was a Wheatstone bridge—in one arm of which is placed the subject, and in the other a resistance box, so that the resistance of the subject can be read directly without any subsequent calculation.

The electrodes used were zinc discs covered with blotting paper soaked in $ZnSO_4$. The galvanometer excursions were photographed.

Experiment 1.—On November 3, two pieces of 1 per cent. belladonna plaster were put on the palm of the left hand and forearm respectively. On November 8 the plaster was taken off, and the subject put into the circuit with the electrodes placed where the plaster had been. The stimulus given was a single sharp blow.

The electrodes were then placed on the normal right palm and forearm, and the same stimulus applied. The results obtained from the two hands did not differ appreciably from one another.

Experiment 2, November 16.—At 2.25 P.M. the subject was injected in the left palm with 1/200 grain atropine sulphate (0·000325 grm.). At 3.5 P.M. the subject was put into the circuit, and a record was taken.

There was no apparent electrical reponse to three successive stimuli of smelling "xylol." However, it is noteworthy that on this occasion the resistance of the hand was very high, so that any alteration of resistance, due to the stimulus, of less than 1000 ohms would hardly be noticeable with the given sensitivity of the galvanometer. In the record taken of the normal hand, the same stimulus did give an alteration of less than 1000 ohms, but in this case it is quite noticeable, because the initial resistance was so much lower.

In the following experiments the subject was put into the double circuit, which Dr. Waller very kindly worked.

In this apparatus, by adjusting the resistance box and the shunt, so as always to bring the spots of light back to zero, the sensitivity of the two galvanometers can be made almost identical, whatever difference of resistance exists between the two hands.

Experiment 3, November 30.—At 1.25 P.M. subject injected in the left palm with 1/200 grain (0·000325 grm.) atropine sulphate. Subject put into the circuits at 3.15 P.M. Stimulus by a whiff of nitro-chloroform. No difference in reaction to be seen between the atropinised and non-atropinised hands.

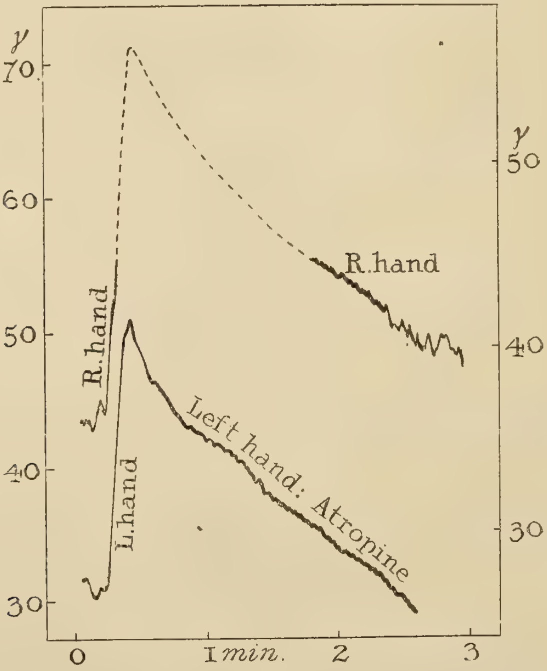

FIG. 6.

Experiment 4, December 12.—At 2.30 P.M. subject injected as before (1/200 grain). At 2.45 P.M. subject put into the circuits. The resistance of the atropinised hand was low at first, but as the atropine began to take effect it became higher and higher. Record taken at 3.15 P.M. Various stimuli were used. The results were negative as in Experiment 3, *i.e.*, emotive responses persisted in both palms.

Experiment 5, January 3.—At 2.0 P.M. the subject was injected in the left palm with 1·5 mgrm. atropine sulphate. At 2.30 P.M. the subject was put into the circuits, various stimuli were used. No appreciable difference was noticed between the response of the atropinised and that of the normal hand.

Experiment 6.—To make quite sure of these results, Miss De Decker very kindly allowed herself to be injected with 2 mgrm. (0·00195 grm.) of atropine sulphate. She was then put into the circuits, and the results were observed by two witnesses. On applying a stimulus, equal responses were obtained from both hands. The drug took full effect, great dryness, parching of the throat, and a headache resulted. (Details of this experiment are given by Dr. Waller, p. 38.)

Conclusions.

I cannot feel that any very definite conclusions can be drawn from these experiments. For instance, the discrepancy between these results and those of Dr. Leva may be due, it seems to me, to one of two reasons.

(1) It may be due to the different effect of atropine on different people—a fact well known to doctors; this could only be decided by carrying out the same experiment on a great number of subjects.

(2) It may be due to technical errors. Dr. Leva does not give any details of his technique, nor does he state whether or not he noted the resistance of his subjects.

As seen in Experiment 2, if the resistance is very high the change due to the stimulus may not be seen on a mediumly sensitive instrument—there should be some means of altering the sensitivity of the galvanometers according to the resistances so as to get all results comparable to one another.

Atropine, in stopping the secretions, makes the resistance higher, so that it is quite possible that in Dr. Leva's experiments the response really took place, but was not detected by the galvanometer.

In these experiments at any rate atropine had no effect on the emotive response.

My best thanks are due to Dr. Waller for all his help.

A Preliminary Study of the Energy Expenditure and Food Requirements of Women Workers.

By O. Rosenheim.

(Communicated by the Roy. Soc. Food (War) Committee. Received April 9, 1919.)

The large share which women have taken during the war in industrial work has brought out the importance of possessing reliable data on which to base a fair assessment of their share in the available food supply, and has also revealed the paucity of our knowledge with regard to the energy expenditure of women workers under factory conditions.*

The food requirements of manual workers are obviously to the largest extent dependent on the energy expenditure of the individual. A scientific system of assessing this expenditure, expressed in calories, may be based on two methods. In the first, which may be called the statistical method, we arrive at the result by indirect means. The actual amounts of the various foodstuffs consumed by a large number of workers are weighed, and from their contents in proteins, fats and carbohydrates, the energy value of the food consumed is calculated. The second method, which may be called the direct method, aims at the direct determination by suitable methods of the energy expenditure of the worker during sleep, recreation, and work. This procedure is *a priori* preferable to the statistical one, but owing to inherent practical difficulties, it has not so far been applied to industrial workers. It is obvious that the complicated and expensive installations necessary for direct calorimetry are unsuitable for work under factory conditions. It was possible, however, to make use in the workshop of the method of indirect calorimetry, the general application of which has been so greatly extended by the introduction of the Douglas bag method.†

An opportunity for carrying out such an investigation was afforded by the establishment of a Training School for Munition Workers at King's College, London, under the direction of Prof. A. H. Jameson, Professor of Civil Engineering. The following report of a research, which was undertaken at Dr. Cathcart's suggestion, as the first of a series, deals with women performing lathe work only. The number of experiments were, unfortunately, not so numerous as was at first intended, owing to the cessation of the activities of the Training School after the declaration of the Armistice. Nevertheless, the close agreement of the values obtained for the "standard" (basal) metabolism (see p. 54) makes the correctness of the other results probable, and justifies the assumption that even a larger number of working experiments would not materially affect the general conclusions.

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* Only in two cases has the energy metabolism of women workers been investigated, namely that of typists, by Carpenter ('Journ. Biol. Chem.,' vol. 9, p. 231 (1911)), and that of women occupied in sewing, housework, washing, etc., by Becker and Hämäläinen ('Skand. Arch. of Physiol.,' vol. 31, p. 198 (1914)). The conditions in both cases were not such as obtain in factories.

† 'Journ. Physiol.,' vol. 63, p. xvii (Proc.) (1911).

1. *Plan of Experiments.*

The general plan of the experiments was to collect data from as many subjects as possible, which would allow the energy expenditure of an average adult woman during 24 hours to be calculated. This period is divided in the everyday life of the worker into three periods of approximately eight hours each, *i.e.*, the periods of sleep, work, and recreation.

(a) *Metabolism during Sleep.*—The energy expenditure during sleep may be assumed, for the purpose of this investigation, to be only slightly smaller than that during complete muscular rest in the post-absorptive condition, *i.e.*, 12–14 hours after the last meal. This value, which might conveniently be termed the "standard" metabolism in preference to the usual term "basal" metabolism* was determined for each subject. The experiments were made at 9 A.M., the subjects coming to work without having partaken of any breakfast, and having rested in a reclining position some time before the experiment. All experiments were made during complete muscular repose, the subjects lying comfortably on a couch.

(b) *Metabolism during Work.*—The following short details about the organisation of the Training School are necessary for the description of the "work" experiments. The school had for its purpose the training of women for the manufacture of aeroplane components, necessitating the use of the lathe. The course extended over eight weeks, of which three weeks were spent in the preliminary shop for general training and five weeks in the aeroshop for work on machines as actually used in the industry. The working hours were from 9 A.M. to 6 P.M. including meal times (12–1 and 4-4.15). Factory discipline was maintained. The workshops were well lighted and heated by radiators. Observations made on several occasions with Hill's kata-thermometer† tend to confirm the subjective impression that the shops were well ventilated and the working conditions comfortable (see Appendix, Table I).

The method of instruction consisted in sets of progressive exercises in both operating and setting.* The operations included parting, recessing, turning (hand-feed), screw cutting, hand-chasing screws, boring (hand-feed and from headstock), etc. Two types of lathes were in use: (1) centre lathes (6″, 7″, and 9″) and (2) Herbert capstan lathes (No. 4 medium and No. 9 heavy). The latter, as used in the manufacturing industrial shops, were supplied by the Ministry of Munitions.

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* See Krogh, 'The Respiratory Exchange of Animals and Man,' London, 1916.

† 'Rep. Loc. Govt. Board,' New Series, No. 100 (1914).

In absence of any simple methods for the actual measurement of work performed, a somewhat arbitrary division became necessary into light, medium, and hard work. The operations of parting, screw-cutting, or chasing, turning, etc., which necessitated merely the exertion of a slight constant pressure during the turning of a handle, were classified as "light" work. Rough turning, boring (hand-feed), and similar operations represented "medium hard" work, whilst operations involving several turns of the capstan or boring heavy work from headstock, etc., were taken to represent "hard" work. The "work" experiments by each subject were not carried out consecutively, but on different days, usually in the afternoon. In each category of work all the subjects performed as far as possible the same operations on the same type of lathe. For the final calculation of the energy output during the eight hours' working period, it was considered, after investigation of the actual conditions, that not more than two hours should be allotted to hard work, and that the mean between "light" and "medium hard" work would fairly represent the remaining six working hours.

(c) *Metabolism during Recreation.*—For various reasons, it is obvious that any estimate of the energy expenditure during the active non-working period can only be approximate. By taking the energy expenditure during walking, standing, and sitting as a basis of calculation, we shall probably err in underestimating rather than in overestimating the bodily activities of the average working girl during recreation hours. As a result of personal enquiries from the subjects of this study, it would seem that these three forms of activities would be fairly apportioned by ascribing 1½ hours to walking, 3½ hours to sitting, and 3 hours to standing. In all cases the energy expenditure during standing was estimated whilst the subject stood in an easy relaxed position, and some time after the performance of any active work. The walking experiments were carried out on the level, the stone-paved corridor of the College (80 m. long) serving as a convenient course, necessitating only one turning movement in a two minutes' walk.

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* The exercises were arranged by the manager of the school, Mr. H. G. Taylor, Lecturer on Engineering, King's College, to whom I am greatly indebted for information and kind help in carrying out this investigation.

2. *Technique.*

The general method of estimating energy expenditure by indirect calorimetry with the help of the Douglas bag is now too well known to require further description.* A bag of about 50 litres content was used. Although a careful test showed that no appreciable loss of CO_2 occurred until after two hours from filling the bag (see Table II, Appendix), the expired air was measured and analysed immediately after each experiment. A Wright's wet meter reading to 1 c.c. was used for the measurement, and the analyses were carried out in Haldane's apparatus. Potassium pyrogallate, as absorbent for oxygen, was found by far preferable in this form of apparatus to sodium hydrosulphite, which is recommended by Durig† for use in the Zuntz-Geppert apparatus. All analyses were made in duplicates, and results showing a difference greater than 5/100 per cent. were discarded and repeated.

In a few preliminary experiments the valve, head and mouthpiece was used as supplied by the makers, Messrs. Siebe, Gorman & Co., Ltd. It became soon evident that this arrangement, excellent as it is for investigation by trained observers on themselves, had serious drawbacks when applied to girl munition workers under factory conditions. The main objections were raised (1) against the nose-clip and mouthpiece, which latter besides being painful to the gums in some cases, gives rise to excessive salivation in all; (2) against the position of the corrugated rubber tubing which coming from the valve passes over the head to connect with the bag. This wide tubing, intersecting the field of vision, interferes seriously with the power of the subject to make the fine adjustments necessary in lathe work.‡ The second objection was successfully overcome by the construction of a new double-acting valve in which the expired air passes downwards. A detailed description of this valve is, however, unnecessary as it still necessitated the objectionable use of the mouthpiece.

It seemed possible to avoid all the above raised objections by employing a suitable face mask, and thus reverting to the principle which was first introduced into the technique of respiration experiments by Edward

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* For full working details see E. P. Cathcart, 'Journ. Roy. Army Med. Corps,' November, 1918.

† 'Biochem. Zeitschr.,' vol. 4, p. 65 (1907).

‡ A further objection, from the investigator's point of view, is against the clicking noise made by the mica discs of the valves which, by attracting the attention of the subject, gives rise to wilful and intentional breathing irregularities. This was easily overcome by using the excellent rubber valves recommended by Y. Henderson, 'Journ. Biol. Chem.,' vol. 33, p. 47 (1918).

Smith* 60 years ago. An excellent face mask which covers the nose and the mouth, leaving the rest of the face exposed, is made by Messrs. Siebe, Gorman and Co., Ltd., for a different purpose.

Firemen's Smoke Mask.—By simply reversing the valves of this apparatus it can be at once adapted for the present purpose, the connecting rubber tubing passing downwards and resting on the shoulder without interfering with the freedom of movement of the subject. It was found advisable to drill an additional series of holes into the metal part of the valves, thus ensuring a perfectly free passage of inspired and expired air. The mask can be easily adapted to any shape of face, owing to the flexibility of the inflated rubber cushion which enables an air-tight fit to be made. There is no doubt a serious difficulty, when using a mask, in obtaining an air-tight fit in men with hairy or sunken cheeks, but as all the subjects of this investigation were young women with smooth and well filled out faces, this difficulty was not met with. The wearing of the mask does not interfere to any extent with the natural rhythm of breathing, as does the mouthpiece and nose-clip. Whereas, with the latter, persuasion was necessary to induce the subjects to carry out the experiments, no objections were raised against the face mask—an important consideration in an investigation in which a satisfactory result largely depends on the good will of the subject. Approximately three-quarters of the experiments were carried out with the help of the face mask, but since some comparative experiments made on myself (see Table III, Appendix), and on one of the subjects gave identical results, it was thought unnecessary to distinguish between them in the following Tables.

3. *Subjects.*

Six young women volunteered as subjects for this investigation. One of them, Subject F, finding manual work too strenuous, returned to her previous occupation (nursing) before the completion of the whole series of observations. Their ages were from 18–29 years and the character of their previous occupation, which is to some extent reflected in their calorie expenditure during work (see later) had been different in each case. Only one of them (Subject A) had 18 months' previous experience of munition work of a different type, a fact which partly explains her small energy expenditure during work (see later). They continued in good health during the time of the observations, care being taken to discontinue the working experiments during the period of catamenia, so as to exclude a possible influence of this factor.* The respiration and pulse rate was recorded in the experiments made in the resting, sitting, and standing positions, but their observation was not found feasible during the working experiments. The diet of the subjects was the ordinary mixed diet in which, under the war conditions then prevailing, the share of carbohydrates may perhaps have been larger than normally. Their meals during working hours were consumed in the refectory provided by the authorities and were probably more or less of uniform character.

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* 'Phil. Trans.,' vol. 149, p. 681 (1859). The remarkable work of this investigator has received a good deal of unjust criticism at the hands of later German workers, whilst many of his original observations have been passed over or were rediscovered (such as the importance of determining the standard (basal) metabolism in the post-absorptive condition, the stimulating influence of proteins on the gaseous metabolism, its increase during walking, and on the treadwheel, etc.).

The energy expenditure per hour and per square metre of body surface has been taken as the basis for comparison of the results,† and in order to enable comparison with previous work the calorie expenditure calculated per kilogramme of body weight per hour has also been included. The area of the body surface was calculated according to the formula of D. and E. F. Du Bois‡ from which a chart was constructed on a scale sufficiently large to read the value to the third place of decimals.

The weight of the subjects (without clothes) was recorded by Mrs. M. C. Rosenheim, to whom I am also indebted for much valuable assistance during the whole course of these experiments. The statistical data of the subjects are given in the following Table:—

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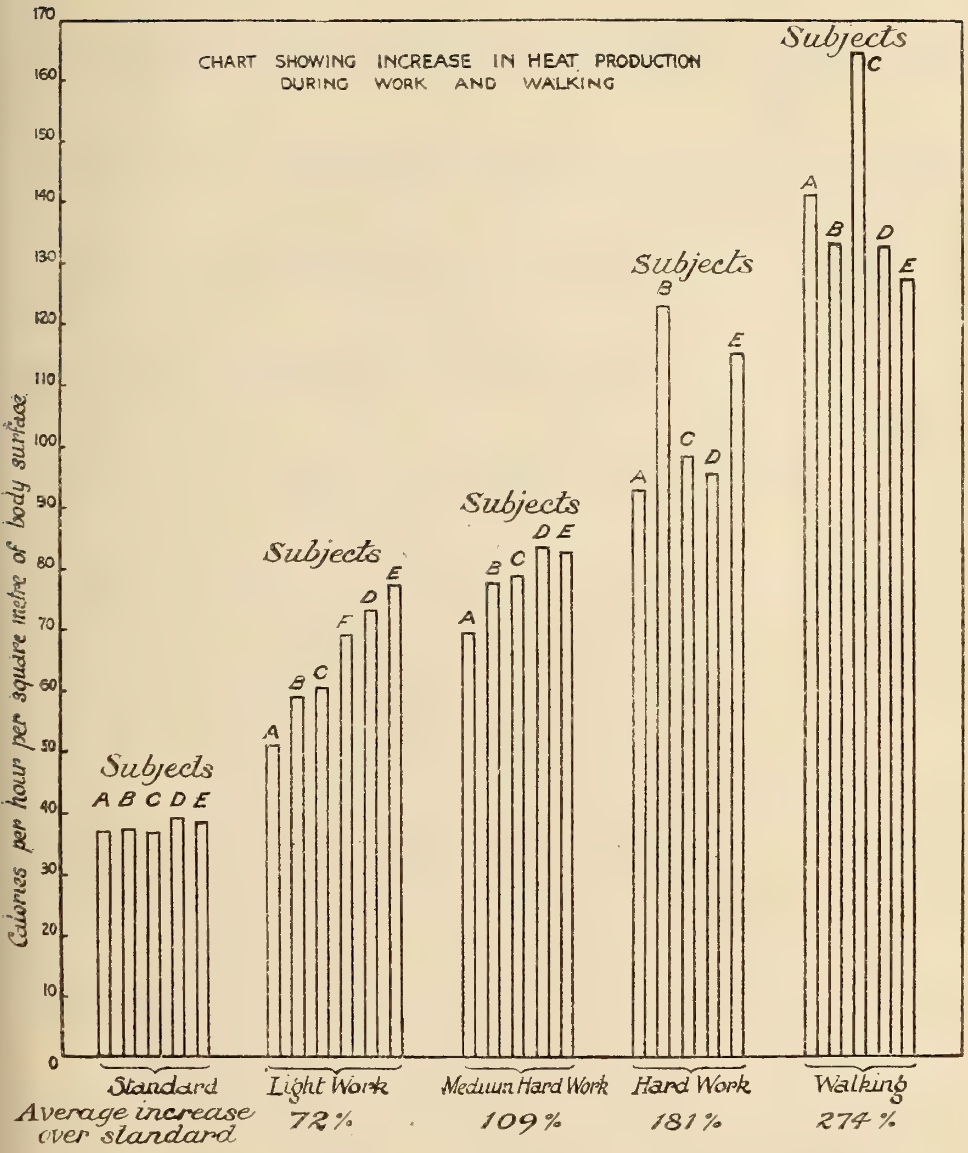
* It has been shown by L. Zuntz, 'Arch. f. Gynäkol.,' vol. 78, p. 106 (1906), that the normal gaseous metabolism is not disturbed by menstruation.

† See G. Lusk, 'Science of Nutrition' (1917), p. 124.

‡ 'Arch. Int. Med.,' vol. 17, p. 863 (1916).

Table I.—Statistical Data of Subjects.

No.	Subject.	Age.	Height.	Weight (without clothes).	Area of body surface.	Previous occupation.	Remarks.
		years.	cm.	kgrm.	sq. metre.		
A	Miss E. Lndr.	22	157·0	57·2	1·566	Cook	Normal, well developed, and muscular. Had 18 months' experience of munition work.
B	Mrs. F. Clvrt.	25	159·5	59·9	1·620	Mill hand	Normal, well developed.
C	Miss M. Ndhm. ...	29	163·0	63·5	1·688	Domestic work	Normal, well nourished and well developed.
D	Miss E. M. Nsh. ...	19	156·5	54·9	1·535	Messenger clerk	Normal, well developed.
E	Miss C. Mrrs.	18	160·8	51·3	1·522	Waitress	Normal, slight build. Had just recovered from three weeks' illness (influenza).
F	Miss A. Wrt.	29	169·4	57·2	1·658	Hospital nurse	Normal, slight build. Found manual work too strenuous and returned to previous occupation.



In the following Table are recorded the results of the experiments on the metabolism during non-working hours:—

Table II.—Metabolism during Non-Working Period.

Sub-ject.	Descrip-tion.	Pulmonary output in litres per minute at 0° and 760 mm.	Pulse.	Respi-ration.	Analysis of expired air.		Carbonic acid eliminated per minute.		Oxygen absorbed per minute.		Respi-ratory quotient.	Calories of heat produced per hour.	
					CO_2.	O_2.	Total.	Per kgrm.	Total.	Per kgrm.		Per kgrm.	Per square metre of body surface.
					per cent.	per cent.	c.c.	c.c.	c.c.	c.c.			
A	Standard	4 ·8	81	10	3 ·81	17 ·05	179 ·8	3 ·15	193 ·1	3 ·38	0 ·93	0 ·96	36 ·7
B	,,	5 ·5	85	20	3 ·02	17 ·44	163 ·5	2 ·73	198 ·2	3 ·31	0 ·83	0 ·98	36 ·8
C	,,	5 ·2	72	16	3 ·53	17 ·00	182 ·0	2 ·87	210 ·2	3 ·31	0 ·87	0 ·97	36 ·5
D	,,	6 ·8	73	20	2 ·85	18 ·02	191 ·2	3 ·48	199 ·0	3 ·63	0 ·97	1 ·09	38 ·9
E	,,	5 ·1	80	12	3 ·89	17 ·15	195 ·9	3 ·82	191 ·0	3 ·72	1 ·03	1 ·13	38 ·2
A	Resting on couch	5 ·0	77	12	3 ·78	17 ·04	188 ·9	3 ·31	197 ·8	3 ·46	0 ·96	1 ·04	37 ·8
B	,,	6 ·2	79	20	3 ·17	17 ·67	193 ·9	3 ·22	203 ·3	3 ·39	0 ·95	1 ·02	37 ·6
B	,,	5 ·7	80	18	3 ·48	17 ·34	195 ·3	3 ·26	203 ·7	3 ·40	0 ·96	1 ·02	37 ·7
C	,,	5 ·7	—	—	3 ·69	17 ·03	205 ·7	3 ·24	225 ·5	3 ·50	0 ·92	1 ·04	39 ·2
C	,,	5 ·3	85	16	3 ·60	17 ·10	190 ·4	3 ·00	208 ·1	3 ·28	0 ·92	0 ·97	36 ·5
C	,,	6 ·0	—	—	3 ·54	17 ·26	210 ·6	3 ·32	222 ·6	3 ·51	0 ·95	1 ·05	39 ·4
D	,,	5 ·7	81	16	3 ·17	17 ·43	180 ·2	3 ·28	206 ·4	3 ·76	0 ·87	1 ·10	39 ·5
E	,,	4 ·6	79	14	3 ·89	16 ·30	182 ·6	3 ·56	206 ·8	4 ·03	0 ·88	1 ·19	39 ·9
F	,,	6 ·3	—	—	3 ·25	17 ·32	201 ·6	3 ·53	231 ·5	4 ·05	0 ·87	1 ·19	40 ·8
F	,,	6 ·0	—	—	3 ·04	17 ·39	181 ·8	3 ·18	222 ·5	3 ·89	0 ·82	1 ·13	38 ·9
A	Standing	5 ·8	95	12	3 ·44	17 ·18	196 ·5	3 ·44	221 ·3	3 ·87	0 ·89	1 ·14	41 ·6
B	,,	8 ·3	93	20	2 ·88	17 ·77	235 ·3	3 ·92	267 ·7	4 ·47	0 ·88	1 ·31	48 ·5
C	,,	7 ·0	87	18	3 ·46	17 ·02	243 ·0	3 ·83	256 ·1	4 ·03	0 ·85	1 ·18	44 ·3
D	,,	7 ·7	100	25	2 ·76	17 ·84	213 ·5	3 ·89	248 ·8	4 ·33	0 ·86	1 ·34	48 ·0
E	,,	5 ·6	97	14	3 ·86	16 ·47	214 ·4	4 ·18	258 ·9	5 ·05	0 ·83	1 ·47	49 ·6
A	Sitting	4 ·4	82	10	4 ·25	16 ·01	185 ·2	3 ·24	223 ·9	3 ·92	0 ·83	1 ·13	41 ·5
B	,,	8 ·3	82	20	3 ·03	17 ·81	241 ·9	4 ·04	249 ·8	4 ·17	0 ·99	1 ·26	46 ·6
C	,,	5 ·5	79	18	3 ·46	16 ·91	190 ·7	3 ·00	232 ·6	3 ·66	0 ·82	1 ·06	39 ·9
D	,,	8 ·3	100	24	2 ·76	17 ·98	226 ·8	4 ·13	250 ·1	4 ·56	0 ·91	1 ·35	48 ·2
E	,,	4 ·9	89	12	4 ·15	16 ·58	201 ·7	3 ·93	227 ·1	4 ·43	0 ·89	1 ·31	43 ·9
			metres per min.										
A	Walking	14 ·6	84 ·2		4 ·67	15 ·92	678 ·8	11 ·88	748 ·4	13 ·10	0 ·91	3 ·88	141 ·4
B	,,	16 ·7	80 ·0		3 ·95	16 ·66	655 ·4	10 ·94	729 ·0	12 ·17	0 ·90	3 ·59	133 ·0
C	,,	19 ·6	78 ·1		4 ·15	16 ·25	809 ·7	12 ·75	949 ·2	14 ·95	0 ·85	4 ·37	164 ·2
D	,,	18 ·9	72 ·7		3 ·42	17 ·36	640 ·0	11 ·66	683 ·0	12 ·44	0 ·94	3 ·71	132 ·7
E	,,	15 ·5	72 ·7		4 ·19	16 ·79	644 ·5	12 ·56	640 ·7	12 ·49	1 ·01	3 ·79	127 ·5

The metabolism during work is shown in the following Table :—

Table III.—Metabolism during Working Period.

Subject.	Description of work.	Type of lathe.	Pulmonary output in litres per minute at 0° and 760 mm.	Analysis of expired air.		Carbonic acid eliminated per minute.		Oxygen absorbed per minute.		Respiratory quotient.	Calories of heat produced per hour.	
				CO$_2$.	O$_2$.	Total.	Per kgrm.	Total.	Per kgrm.		Per kgrm.	Per square metre of body surface.
				per cent.	per cent.	c.c.	c.c.	c.c.	c.c.			
A	Light (turning)	7″ centre	6·6	3·64	16·90	238·5	4·37	273·5	4·79	0·87	1·38	50·6
B	,, (screw chasing)...	6″ ,, ...	9·9	3·41	17·56	318·8	5·32	318·8	5·32	1·00	1·61	59·6
C	,, (parting)	7″ ,, ...	9·1	3·21	17·35	289·4	4·56	345·1	5·43	0·87	1·59	60·1
D	,, (parting)	9″ ,, ...	11·4	3·11	17·74	350·9	6·40	367·2	6·69	0·96	2·00	71·6
D	,, (screw chasing)...	7″ ,, ...	12·3	2·95	17·84	358·7	6·54	385·2	7·02	0·93	2·09	74·7
E	,, (screw cutting)...	7″ ,, ...	11·6	3·34	17·55	384·9	7·50	406·9	7·93	0·95	2·37	79·9
E	,, (recessing).........	7″ ,, ...	10·9	3·43	17·41	369·7	7·21	386·3	7·53	0·96	2·26	76·0
E	,, (screw chasing)...	7″ ,, ...	12·2	3·24	17·73	392·6	7·65	392·6	7·65	1·00	2·32	78·1
E	,, (turning)	No. 4 capstan	11·3	3·27	17·70	366·9	7·15	366·9	7·15	1·00	2·17	73·0
F	,, (parting)	7″ centre	10·2	3·61	17·24	365·2	6·39	379·6	6·64	0·96	1·99	68·6
F	,, (turning)	7″ ,, ...	10·7	3·54	17·35	367·7	6·59	386·4	6·76	0·98	2·03	70·2
F	,, (screw cutting)...	7″ ,, ...	10·8	3·28	17·45	351·0	6·14	382·4	6·69	0·92	1·98	68·4
A	Medium hard (boring)	7″ ,, ...	9·4	3·69	17·13	344·4	6·03	361·4	6·32	0·95	1·89	69·1
A	,, ,, (rough turning)	7″ ,, ...	9·3	3·62	17·06	334·8	5·86	367·9	6·44	0·91	1·91	69·8
B	,, ,, (turning)	7″ ,, ...	11·9	3·31	17·63	391·7	6·54	396·8	6·62	0·99	1·99	73·9
B	,, ,, (boring)	7″ ,, ...	12·4	3·54	17·42	435·2	7·27	435·2	7·27	1·00	2·19	81·3
C	,, ,, (turning)	7″ ,, ...	11·1	3·48	17·15	382·9	6·03	430·7	6·78	0·89	2·00	75·4
C	,, ,, (boring)	No. 4 capstan	14·6	3·80	16·77	412·0	6·49	466·1	7·35	0·88	2·15	81·1
D	,, ,, (boring)	7″ centre	13·7	2·98	17·88	405·2	7·38	422·9	7·71	0·96	2·31	82·6
D	,, ,, (rough turning)	7″ ,, ...	13·9	2·92	17·82	402·8	7·34	441·6	8·05	0·91	2·39	85·3
E	,, ,, (boring)	9″ ,, ...	11·9	3·49	17·47	413·6	8·07	413·6	8·07	1·00	2·44	82·3
A	Filing (60 strokes per minute)	—	12·8	4·12	16·88	525·4	9·19	518·6	9·08	1·01	2·75	100·5
A	Hard (12 turns of capstan)	No. 4 capstan	11·6	3·74	16·81	433·2	7·58	493·4	8·63	0·88	2·54	92·6
B	,, (boring)	No. 9 capstan	17·5	3·65	17·12	632·8	10·56	675·3	11·27	0·94	3·44	124·7
C	,, (boring from headstock	9″ centre	14·7	3·53	17·19	513·8	8·09	558·5	8·80	0·92	2·61	98·1
D	,, (rough turning, hand-feed)	9″ ,, ...	15·0	3·16	17·59	471·0	8·59	511·4	9·32	0·92	2·77	98·9
D	,, (10 turns of capstan)	No. 4 capstan	13·9	3·05	17·52	449·3	8·19	487·5	8·88	0·86	2·59	92·9
E	,, (boring)	No. 9 capstan	15·0	3·66	17·04	542·9	10·58	592·3	11·55	0·92	3·43	115·4

Discussion of Results.

(a) *Standard Metabolism.*—An examination of the Tables brings out a remarkable agreement in the figures for the standard metabolism *inter se* and with the results in the literature so far as they can be used for comparison. The investigations of Benedict and Emmes,* in which previous work is also discussed, supplies the largest amount of material for comparison, containing the results of experiments on 68 women. By applying Du Bois formula to the data available F. C. Gephart and E. F. Du Bois† give 37 calories per hour per square metre (with ± 10 per cent. maximum variation from the mean) as the average "basal" heat production of adult women between the ages of 20–50 years. The average of the five experiments above described is 37·4 calories with a maximum variation of from $-2·5$ per cent. to $+4$ per cent., using the Du Bois formula. Considering that these results were obtained by different methods, the agreement is sufficiently close to furnish a further confirmation of the claims made for the contention that the unit of surface area eliminates the same amount of heat in the normal adult, the figure for women being about 7 per cent. lower than that for men. As equal care to guard against all avoidable errors was taken in the subsequent experiments on work, it may be assumed that they are at least equally correct, and that their relatively small number does not materially affect the reliability of the general conclusions drawn from them.

(b) *Metabolism during Non-working Hours.*—The figures obtained in the resting, but not post-absorptive condition, are mainly of interest as showing how little the metabolism under these conditions differs from the standard metabolism. As the experiments were usually made from 3–4 hours after a light breakfast, the stimulating effect of the food ingestion had already passed off to a large extent. Subjects D and E show somewhat higher results than the others in the "sitting" and "standing" experiments, but since the metabolism of these subjects seems to be generally on a higher plane, this result is not remarkable. The pulse rate of Subject D happened in these experiments to be unusually high, but its relationship to the respiration rate together with the absence of any other symptoms do not justify the assumption that her condition was not what would usually be called "normal."

The energy expenditure of women during walking has not so far formed the subject of any investigation, and the amplified analysis of the "walking" experiments as shown in the following Table may, therefore, not be out of place:—

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* 'Journ. Biol. Chem.,' vol. 20, p. 253 (1915).

† 'Arch. Int. Med.,' vol. 17, p. 902 (1916).

Table IV.—Analysis of Walking Experiments.

Subject.	(*a*) Weight (with clothing). kgrm.	(*b*) Velocity. Metres per min.	(*c*) Horizontal kgrm.-metre. (*a* × *b*.)	(*d*) Increase of heat output during walking (= Walking − Standard). Calories per min.	Energy requirement for moving horizontally 1 kgrm. body-weight for a distance of 1 metre.	
					Grm. cal. (*d* ÷ *c*).	Kgrm.-metre.
A	59·7	84·2	5·027	2·73	0·543	0·231
B	62·4	80·0	4·992	2·62	0·525	0·224
C	66·0	78·1	5·155	3·59	0·696	0·294
D	57·4	72·7	4·173	2·39	0·573	0·244
E	53·8	72·7	3·911	2·27	0·580	0·247

The subjects walked at an easy ordinary rate which varied in the different individuals from 73 to 84 metres per minute (2·7 to 3·1 miles per hour). The so-called "maximal economic velocity" in horizontal walking for men is at the rate of 80 to 85 metres per minute. Judging by the figures available in the literature, which need not here be gone into, the results show that women expend practically the same amount of energy as men in moving horizontally 1 kilo. body weight through a distance of 1 metre.

The results when represented graphically (see Chart) illustrate the variations which are usually found in different subjects during walking and also the effect of occupational training. This is most obvious in the case of Subject E, whose low total energy expenditure in walking may be directly traced to the training received in her previous occupation as waitress.*

The chart further reveals the interesting fact that the energy expenditure during one hour's horizontal walking is in all cases higher than that of even the hardest work on the lathe.

(c) *Metabolism during Work.*—The following Table (Table V) contains a compilation of the data for the total metabolism per hour and its increase over the standard metabolism due to various forms of work. The last column shows the relative increase, taking the standard metabolism as the unit. An inspection of the chart, representing the results in a graphic form and arranged according to the calorie expenditure of the individual subjects, shows that during "light" work their energy expenditure stands in a certain relationship, which is still maintained in the same order during "medium hard" work. During "hard" work, however, the energy expenditure of the

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* It is interesting to note the general agreement of these figures with the old experiments of Edward Smith on himself (*loc. cit.*), who found an increase of 2·5 to 3·5 times his basal metabolism, when walking horizontally at the rate of from 2 to 3 miles per hour.

Table V.—Metabolism per Hour and Increase during Work (compared with Standard Metabolism)

Subject.	Description.	CO_2 eliminated.		O_2 absorbed.		Heat produced in calories.			Work performed.*	Heat increase. (Standard = 1.)
		Total.	Per sq. m.	Total.	Per sq. m.	Total.	Per kgrm.	Per sq. m.		
		grm.	grm.	grm.	grm.				kgrm.	
A	Standard	21·2	13·5	16·6	10·6	57·5	0·96	*36·7*	—	1·00
A	Light work	28·1	17·9	23·5	15·0	79·2	1·38	*50·6*	1,900	
	Increase	6·9	4·4	6·9	4·4	21·7	0·42	13·9	—	*0·38*
A	Medium hard work†	40·0	25·5	31·3	20·0	108·6	1·90	*69·5*	4,570	
	Increase	18·8	12·0	14·7	9·4	51·1	0·94	32·8	—	*0·89*
A	Hard work	51·1	32·6	42·3	27·0	144·9	2·54	*92·6*	7,600	
	Increase	29·9	19·1	25·7	16·4	87·4	1·58	55·9	—	*1·52*
A	Metal filing	61·9	39·5	44·5	28·4	157·3	2·75	*100·5*	9,320	
	Increase	40·7	26·0	27·9	17·8	99·8	1·79	63·8	—	*1·74*
A	Walking 3·1 miles per hour	80·0	51·1	64·2	41·0	221·5	3·88	*141·5*	14,530	
	Increase	58·8	37·6	47·6	30·4	164·0	2·92	104·7	—	*2·85*
B	Standard	19·3	11·9	17·0	10·5	58·4	0·98	*36·8*		
B	Light work	37·6	23·2	27·3	16·9	96·5	1·61	*59·6*	3,860	
	Increase	18·3	11·3	10·3	6·4	38·1	0·63	22·8	—	*0·62*
B	Medium hard work†	48·8	30·1	35·7	22·0	125·8	2·09	*77·6*	6,520	
	Increase	29·5	18·2	18·7	11·5	67·4	1·11	40·8	—	*1·11*
B	Hard work	74·6	46·1	57·9	35·7	201·4	3·44	*124·7*	13,100	
	Increase	55·3	34·2	40·9	25·2	143·0	2·46	87·9	—	*2·38*
B	Walking 2·98 miles per hour	77·3	47·7	62·5	38·6	215·3	3·59	*133·0*	14,120	
	Increase	58·0	35·8	45·5	28·1	156·9	2·61	96·2	—	*2·61*

C	Standard	21·5	12·7	18·0	10·6	61·6	0·97	*36·5*		
C	Light work	34·1	20·2	29·6	17·5	101·4	1·59	*60·1*	3,320	
	Increase	12·6	7·5	11·6	6·9	39·8	0·62	23·6	—	*0·65*
C	Medium hard work†	46·9	27·8	38·5	22·8	132·0	2·08	*78·2*	6,270	
	Increase	25·4	15·1	20·5	12·2	70·4	1·11	41·7	—	*1·14*
C	Hard work	60·6	35·9	47·9	28·4	165·6	2·61	*98·1*	9,400	
	Increase	39·1	23·2	29·9	17·8	104·0	1·64	61·6	—	*1·68*
C	Walking 2·9 miles per hour	95·5	56·6	81·4	48·2	277·2	4·37	*164·2*	18,750	
	Increase	74·0	43·9	63·4	37·6	215·6	3·40	127·7	—	*3·50*
D	Standard	22·5	14·7	17·1	11·1	59·7	1·09	*38·9*		
D	Light work†............	41·8	27·2	32·3	21·0	112·3	2·05	*73·2*	4,710	
	Increase	19·3	12·5	15·2	9·9	52·6	0·96	34·3	—	*0·88*
D	Medium hard work†	47·6	31·0	37·1	24·2	128·8	2·35	*83·9*	6,150	
	Increase	25·1	16·3	20·0	13·1	69·1	1·26	45·0	—	*1·16*
D	Hard work	54·3	35·4	42·9	28·0	147·3	2·68	*95·9*	7,870	
	Increase	31·8	20·7	25·8	16·9	87·6	1·59	57·0	—	*1·47*
D	Walking 2·7 miles per hour	75·5	49·2	58·6	38·2	203·6	3·71	*132·7*	12,890	
	Increase	53·0	34·5	41·5	27·1	143·9	2·62	93·8	—	*2·41*
E	Standard	23·1	15·2	16·4	10·8	58·1	1·13	*38·2*		
E	Light work‡............	44·6	29·3	33·3	21·9	116·8	2·28	*76·8*	5,240	
	Increase	21·5	14·1	16·9	11·1	58·7	1·11	38·6	—	*1·01*
E	Medium hard work...	48·8	32·1	35·5	23·3	125·2	2·44	*82·3*	6,080	
	Increase	25·7	16·9	19·1	12·5	67·1	1·31	44·1	—	*1·15*
E	Hard work	64·0	42·0	50·8	33·4	175·6	3·43	*115·4*	10,300	
	Increase	40·9	26·8	34·4	22·6	117·5	2·30	77·2	—	*2·02*
E	Walking 2·7 miles per hour	76·0	49·9	54·9	36·1	194·0	3·79	*127·5*	12,420	
	Increase	52·9	34·7	38·5	25·3	135·9	2·66	89·3	—	*2·33*

* Average of results calculated from the increase of CO_2 and O_2. The factors determined by Benedict and Carpenter (Carnegie Institution Publications, No. 126, 1910) were used.

† Average of two experiments.

‡ Average of four experiments.

different subjects is more variable, but, in all forms of work, Subject A, probably owing to her previous training as munition worker, performed the work with a minimum expenditure of energy. The work economy of this subject is also seen in the experiment in which she performed the relatively strenuous work of metal filing (60 strokes per minute), and in which her energy expenditure only rose slightly, over that of the hardest work on the lathe (see Table III). The general results of the work experiments lead to the conclusion, which might not *a priori* be expected, that the energy required for lathe working is relatively small.

The Daily Food Requirements of Lathe Workers.

By means of the data obtained, although they are not perhaps sufficiently numerous to warrant final conclusions, we may arrive at an approximate estimate of the food requirements, expressed in calories, of the women who formed the subjects of this inquiry. The following Table (Table VI) shows the energy expenditure of the subjects in the three periods of sleep, work, and recreation, calculated on the basis of the considerations set forth in the introduction. The sum of these, multiplied with the figure for the total body surface of the subject in each case, represents what might be termed the "net" calorie requirements, and an increase of 15 per cent. on this figure represents the corresponding calorie values of the food as purchased.

It will be seen that the final figures show that the calorie values of the food required by women engaged on lathe work vary from 2400 to 2800 calories. It is interesting to compare this result with the recommendations made by L. E. Hill and his co-workers, based on their statistical nquiries.* For a man on moderate munition work, 3000–3500 calories per day were suggested as a standard, the corresponding figure for women being 2400–2800 calories. The absolute agreement of the figures obtained by these two inquiries, based on entirely different methods, seems to be more than an accidental coincidence. It is hardly necessary to point out that, in assessing the food requirements of women workers, due consideration must be paid not only to the quantitative side, as dealt with above, but also to the qualitative side, in respect to the presence in adequate amounts of the all-important accessory factors or vitamines.

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* See Memorandum No. 19, 'Health of Mun. Work. Com.,' 1917.

Table VI.—Food Requirements (in Calories) per 24 Hours.

		Miss Lndr.		Mrs. Clvrt.		Miss Ndhm.		Miss Nsh.		Miss Mrrs.	
8 h. sleep (=8 × standard metabolism)			293 ·6		294 ·4		292 ·0		311 ·2		305 ·6
8 h. work	2 h. hard work	185 ·2	545 ·8	249 ·4	678 ·0	196 ·2	629 ·4	191 ·8	663 ·4	230 ·8	708 ·4
	6 h. average of light and medium work	360 ·6		429 ·6		433 ·2		471 ·6		477 ·6	
8 h. recreation	1 ·5 h. walking	212 ·1	482 ·2	199 ·5	508 ·1	246 ·3	518 ·9	194 ·1	506 ·8	191 ·3	493 ·8
	3 ·5 h. sitting	145 ·3		163 ·1		139 ·7		168 ·7		153 ·7	
	3 h. standing	124 ·8		145 ·5		132 ·9		144 ·0		148 ·8	
Total per sq. m. of body surface		1321 ·6		1480 ·5		1440 ·3		1481 ·4		1507 ·8	
Net calories*		2070		2400		2430		2275		2300	
Gross calories†		**2380**		**2760**		**2800**		**2620**		**2640**	

* = calories for total body surface.

† = Net calories + 15 per cent. increase.

APPENDIX.

Table I.—Kata-thermometer Observations.

Place.	Kata thermometer time in seconds for fall from 37 ·8°–32 ·2° C.		Rate of heat loss (per second) of 1 sq. cm. surface at 36° C., grm. cal.		Thermometer.		Relative humidity.	Barometer.	Remarks.
	Wet.*	Dry.*	By radiation, convection, and evaporation.	By evaporation.	Wet bulb.	Dry bulb.	Per cent.	mm.	
Aero-shop, centre	32	75	16 ·2	9 ·3	14 ·2	17 ·5	68	761 ·2	Engine going, five ventilators open.
Preliminary shop, near door	26	58	19 ·9	11 ·0	12 ·9	15 ·1	76	761 ·2	Engine going, one ventilator open.
Preliminary shop, centre	29	70	17 ·8	10 ·4	13 ·0	15 ·0	78	761 ·2	Engine going, one ventilator open.
Same day on roof of King's College	14	19	36 ·9	9 ·7	10 ·2	11 ·9	80	761 ·2	Cloudy, S.W. breeze.
Aero-shop, centre	68	173	7 ·6	4 ·7	14 ·5	17 ·5	70	762 ·5	Engine going, one ventilator open.
Same day on roof of King's College	27	48	19 ·2	8 ·4	10 ·0	12 ·0	77	762 ·5	Dull, W. wind.

* Average of three readings.

Table II.—Test of Douglas Bag.

The expired air was collected during a period of five minutes from a subject sitting on a chair. Volume 31·4 l. (corr.). Samples were taken from the bag and analysed in the intervals given in the Table. The subject's body-surface area was 1·72 sq. m. (height = 166·5 cm., weight = 64 kgrm.).

Analysis of Expired Air.

Time.	CO_2.	O_2.	N.
h.	Per cent.	Per cent.	Per cent.
0	4·015 3·993 } 4·00	16·75 16·75 } 16·75	79·25
1	3·916 3·919 } 3·92	16·77 16·74 } 16·75	79·33
2	3·871 3·865 } 3·87	16·72 16·74 } 16·73	79·40
5	3·826 3·835 } 3·83	16·73 16·73 } 16·73	79·42
22	3·456 3·474 } 3·47	16·87 16·90 } 16·89	79·64

The following table shows that the final result is not appreciably affected until after two hours from the beginning of the experiment :—

Time.	R.Q.	CO_2 eliminated per min.	O_2 absorbed per min.	Calories of heat produced per hour.		Error.
				Per kgrm.	Per sq. m.	
h.		c.c.	c.c.			Per cent.
0	0·93(7)	249·3	266·1	1·23(9)	46·15	—
1	0·91(4)	242·3	265·2	1·24(4)	46·29	+0·30
2	0·89(4)	241·2	269·9	1·24(4)	46·28	+0·28
5	0·88(8)	238·6	268·8	1·21(5)	45·09	−2·30
22	0·81(9)	216·0	263·7	1·19(1)	44·32	−3·96

Table III.—Comparison of Face Mask and Mouthpiece.

	Pulmonary output, litres per min.	Pulse.	Respiration.	Analysis of expired air.		CO_2 eliminated per min.	O_2 absorbed per min.	R.Q.	Calories of heat produced per hour.	
				CO_2.	O_2.				Per kgrm.	Per sq. m.
				per cent.	per cent.	c.c.	c.c.			
Mouthpiece	6·4	67	10	4·02	16·93	258·2	248·9	0·99	1·22	45·6
Face mask ...	6·0	74	11	4·08	16·67	241·9	257·9	0·94	1·20	44·7

The experiments were made on different days. The subject was sitting on a chair. Subject's body surface 1·72 sq. m. (weight 64 kgrm., height 166·5 cm.). To save space only two of six similar experiments are given.

Report on the Metabolism of Female Munition Workers.

By M. Greenwood, C. Hodson, and A. E. Tebb.

(Communicated by E. H. Starling, F.R.S. Chairman of Food (War) Committee. Received April 29, 1919.)

Introduction.

The research described in this report was planned on the following lines:—

In October, 1918, portable Haldane analysers and other apparatus were installed in the Research Sub-section of the Welfare and Health Section of the Ministry of Munitions, and the three authors carried out a considerable number of determinations upon themselves, both with the object of acquiring or regaining familiarity with the experimental technic and in order to determine what form of respiratory apparatus would be most suitable for use in the study of factory conditions. Ultimately, a system was evolved which conforms to the rules afterwards laid down for the guidance of investigators by the Metabolism Research Sub-Committee of the Royal Society Food (War) Committee.

Having acquired the necessary experience, it was decided to proceed on the following general lines:—Permission having been granted to make observations in a national projectile factory in the north-east of London, we arranged to make measurements upon women engaged on the various operations of the factory, while attention was coincidently directed to the cooling power of the air in the workrooms; or, more exactly, in each experiment on a worker, the cooling power of the air was ascertained in a position as near as possible to the worker during the experiment, that is to say, for the purpose in view, practically in an identical position. The respiration apparatus used was a modification of the Douglas bag combined with a face mask (approved by the Metabolism Research Sub-Committee), and needs no special description.

The measurements in the factory were made by A. E. T. and C. H., who were also responsible for the gas analysis. M. G. and his statistical assistants have reduced the analytical readings, the method being that described in Cathcart's recent paper.*

As advised by the Sub-Committee, air analyses (of the room air) have been made at frequent intervals to check the efficiency of the apparatus. In a later section some remarks on the experimental error will be made; here it is sufficient to observe that the analytical accuracy of the work of C. H., upon whose results the greater part of the calculations of the energy values depend, was tested in a combined experiment, samples of the same expired air being analysed by Mrs. Hodson, Miss Hargood Ash, and Dr. M. S. Pembrey. The agreement between these three workers was sufficiently close to make it probable that the conclusions here detailed are trustworthy within the limits implied or expressed throughout the paper (*vide infra*).

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* 'Journal Royal Army Medical Corps,' November, 1918.

Section 1 contains a general description of the factory conditions. Section 2 deals with the salient features of individual processes studied; in Section 3 the results are criticised from the experimental point of view; in Section 4 the interpretation of the figures in summary form is taken up; and in Section 5 the economic implications of the results are briefly discussed.

Section 1.

The whole of the experiments were carried out during November, December, January, February, and March, 1918–19, in a factory manufacturing 6-inch shell cases. At the time when the experiments began, the output of the factory was small; the efforts then being made to speed up production were relaxed owing to the Armistice, the number of workpeople was reduced by more than, and the individual output by at least, 50 per cent. Consequently, the atmosphere of the factory has changed, and, during our observations, there was no hurry or bustle, no cumulative fatigue effects.

An attempt was made to stimulate the workpeople volunteering for respiration tests to work up to their piece-work speed during each experiment, but this cannot have sufficed to reproduce the earlier conditions. Perhaps it would be fair to say that the circumstances more closely resembled those of ordinary industrial life, at least in trades the average earnings from which are above the margin of subsistence, than would have been the case had we carried out the research a few months earlier.

The atmospheric conditions experienced by a worker in this factory varied widely from place to place as regards temperature and moisture—ranging at the same moment from practically those of the open air at the time to those of uncomfortable warmth and stuffiness according to the position chosen. The operations also presented a widely contrasting range of types of work.

Two factors, which made largely for success in experimenting in this factory, were, firstly, the cordiality with which the staff assisted the investigators, moved in many instances by a genuine interest in the enquiry; and, secondly, the fact that one of us (A. E. T.) had already spent some time in the shops, had won the confidence of all the shop managers and of a great many of the charge hands, and was popular with the operatives, who, encouraged by him, volunteered much more readily for the tests than might otherwise have been the case.

The factory has three main parts, the press-house, or forge, and two great shops. The press-house consists of a shed with one side open to a level of 12 feet, the general dimensions being some 100 by 26 yards superficially, with an elevation of 10 yards. There are seven furnaces, placed near the centre, with eight adjacent presses. Both on account of this construction and the use for which it is designed, the general temperature of and the local temperatures in this press-house may all vary enormously. At times of great pressure, *with a still air outside,* it becomes, even in cold weather, terrifically hot (for with all the furnaces busy, and an accumulation of rough forgings in all states of heat nearly covering the floor, sources of radiant and convected heat have become, after a few hours' work, very numerous), and the heat escapes but slowly into still outside air, even through the open side of the press-house. On the other hand, with fewer forgings the general heat production will be much less, and also with certain winds, even though forgings be numerous, an icy blast will sometimes sweep the place from end to end, and cooling will in consequence be very rapid. For similar reasons, the local temperatures in this forge at one and the same moment are subject to wide variations.

The two great *workshops* are alike in structure and arrangements; both contain the machinery requisite for completing the shell case. The length of each shop is over 200 yards, portions being screened off at one side to make tool-rooms and stores. Lighting, heating, and ventilation are from overhead, but a free circulation of air is aided by numerous doors at the sides and sliding panels at the ends. The heating apparatus is quite inadequate, pipes being placed so high as to have little noticeable effect in the body of the shop. In cold weather it has to be supplemented by large coke fires in pails. Closed rooms for the warm processes (varnishing and pickling) are partitioned off at one end of the main shop. Lathes occupy two-thirds of the floor space, the largest for rough turning, and diminishing in size down to the small models used for fashioning base plates. Near the centre of the shop are a few nosing furnaces (for shaping the shell head), and others serve to heat the copper bands, which are fitted to the cases in a hydraulic press. There are also two small cabins, where the inner surface of the shell is sandblasted.

The system of overhead "runs" for lifting and moving the shell to and from the lathes is carried throughout the building, and at the ends the lathes give place to benches for gauging and assembling the cases.

The atmosphere of the tool room and that of the varnishing room deserves comment. In the former, where high speed steel is being ground on corundum wheels and rockers, there is an odour of burning, in the latter the air is warm and feels stuffy, possibly on account of fumes generated by the gas heaters used for ovens and cauldrons. The temperature of this room varies considerably at different times, both on account of the stoves in it, and of the piles of cooling shells removed from them (after their varnished interiors have been sufficiently baked), as well as from the fact that two large doors at one end are periodically thrown open wide to let out a trolley full of shells, when the temperature suddenly falls, it may be, many degrees.

Section 2.

Such being the general atmosphere of the factory, we pass to the relevant details of the several operations studied.

The Press-house.—The work of the press-house is generally regarded as the most arduous in the factory. Men work the forges and remove the cooled forgings; the intermediate process is carried out by gangs of three women, viz., the press driver, the tonger, and the ringer.

The press driver, standing on a small platform, works the press by means of three levers; a pedal for the right foot releases one; the handles are at about elbow height, and the levers are pulled and pushed, using the weight of the body which is thrown as far forward as possible, and the degree of pressure has to be exactly regulated. The press driver also keeps the press cooled by turning on water after each forging and periodically steps down to tar the mould and punch.

The tonger moves the yellow-hot billet from the forge table and drops it into the press, from which she afterwards lifts the forging and tips it on to the ground. The tongs are about 5 feet long and are suspended by a wire from the overhead run which bears both the weight of billet and tongs, and the chief exertion consists first in applying the necessary grip, at the moment of raising the billet from the table, and again when the last few inches of the forging are levered out of the press (it being automatically pushed through about five-sixths of the mould after the punch has been withdrawn), the body weight is thrown on to the tongs to give leverage, this being necessary because the forging is practically never raised quite to the mouth of the mould.

The ringer drops one or more iron rings on to the billet (which increases the depth of the mould and prevents the rising edges of the forging sailing over outwards) and removes them as the forging rises out of the mould. She also throws coal dust upon the hot billet and trundles the hot forging away after it is tipped off the press on to the floor.

Under the present conditions of slack work there are many intervals between sets of forgings; usually six to nine billets are drawn out of the forge in series and then there is a pause. Forty billets in the hour is an average piece rate; in one of our early experiments seven forgings were turned out in 5 minutes 12 seconds. These billets weigh about 150 lb. apiece.

Lathe Work.—In classifying our experimental results we have grouped lathe workers as on "heavy turning," on "turning" without a qualifying word, or on "light turning." This classification does not correspond exactly with an ordinary factory nomenclature. In our analysis "heavy" denotes shell turnings only, and applies to the first rough turning when the shell head has not yet been shaped, and a clutch adjustment is required for fixing the object, so that both fixing and removing are laborious. *Turning* includes both base-facing (where a clutch is used), and those later processes on the shell where fixing is done merely by screwing. *Light turning* only includes the turning of gas containers: (*a*) the rough turning on first process; (*b*) the boring done on a small capstan lathe; (*c*) the finishing operation. Of these three operations the first involves very little work, the lathe, once set, runs for some time and little measurement is necessary; in boring, the full weight of the body is sometimes brought to bear upon the wheel to drive the tool home in the internal finishing; the final processes are very exact, the lathe tools being often changed, while gauging is repeated at very frequent intervals, and the task is described as "worrying" work.

In these lathe operations the temperamental factor is probably more important than in any others. While the body of the shell or of the gas container is being machined, there is a period of "standing to" (longest in heavy turning and correspondingly reduced when smaller units are in question). A quick and eager worker will fill up all this interval with small movements, *e.g.*, gauging, cleaning portions of her machine, sweeping off swarf, preparing for the next job, and so forth. A phlegmatic worker may sit quietly waiting. This fact emphasises the contention made later on that very long series of observations are needed to reach a correct average value.

Banding.—(Band Turning). Is very accurate work, each stage of the operation lasts barely three seconds, the whole but three or four minutes while gauging is constant and exacting. The shell is held on the lathe by a false bottom, the end plate, which has to be hammered on before the turning is done and hammered off afterwards. There is no waiting in this work.

Cleaning and Drying.—The shell case is rolled to and fro upon a bench, at elbow height, with one hand while the interior is swabbed with a mop held in the other hand. The woman stands sideways to the table and rolls the shell 2½ to 3 feet each way, the weight of the body being thrown from one foot to the other with a swinging motion.

Shell Hoisting.—This is a part of the copper banding process, a clutch is put into the mouth of the shell, attached to a rope passing over a pulley on the overhead run, and the shells are lifted into the banding press, out again and then across the gangway to the next bench.

We may here remark that a female worker often moves heavy shells from one position to another but always by means of mechanical aid. In the press-house the suspended tongs acts as a lever, while at the lathes the shell-lifter is only another form of pulley allowing the shell to be gripped in a horizontal position.

Tool Setting.—There are all forms of grinding and some exertion was evidently needed in using one or two stiff machines, especially when the worker so set the stone as to give a maximum amount of friction. In all machines there were two adjusting wheels one for each head. On the Gisholt machine it is only the stone which is fixed, the machine (which grips the tool) swinging backwards and forwards against it. File grinding appeared to need fine adjustment of every movement and both hands were used; the friction did not seem great.

The value of observations in this department is reduced by the notorious idleness of the operatives after November 11. Often the work under experimental study was all the work done in the hour.

Gauging or Examining.—Three types of work are covered by this heading: (*a*) The large shell cases are rolled to and fro horizontally while calipers test the thickness of the walls. When this is over the shell is stood upright and tipped over to the opposite side of the bench. The ordinary method is to bend the knees, drop the elbows and, gripping the shell mouth with both hand, to spring slightly upwards, thus raising the weight without strain. (*b*) Gauging the tops of gas containers: spring bungs are removed before gauging and hammered in afterwards, the examiner stooping to pick them up from the ground. This work is done while seated. (*c*) The final testing of gas containers: here the shell stands upright on the bench and a screw gauge is screwed in and out, the whole container is screwed loose from the shell and the shell shunted back upon the bench; the hands are working on a level with the chin.

Stamping.—Marks are cut upon the sides and ends of the shells as they lie upon the bench. The stamp resembles a short cold chisel, of which the sharp end is the stamp to be impressed; it is given a smart blow with a heavy hammer.

The ends of the shell are approached in an uncomfortable stooping attitude, there are many dies in the stamp box but the right one is rapidly selected. The marks to be stamped are chalked on the shell and great accuracy is needed. The rate is seven blows in 10 seconds.

Labouring (Sweeping and Barrow).—Steel filings are removed from trays under the lathes with large shovels and placed in iron barrows, the loaded barrows are then wheeled away and the contents dumped outside the shop. We have also included ordinary rubbish sweepings, which seemed to be equally laborious work. This is the only type of work seen to produce sensible perspiration and, as will appear, the experimental results confirm the opinion that a lightly regarded and relatively ill-paid task is physiologically the most costly of all.

Walking and Carrying.—The few observations under this heading refer to persons carrying such objects as boxes of bullets, a tea tray, gas containers, etc. In no case was the load heavy; perhaps of the order of an ordinary coal scuttle or a marketing basket.

Section 3.

At this point it will be advisable to touch upon the material accuracy of our experiments, merely *as* experiments, quite apart from the interpretation of experiments deemed to be in themselves exact.

When the whole volume of expired air is analysed, as for instance in the research of Benedict and Cathcart upon the metabolism of cyclists, the only error involved is due to inexact graduation (fineness of the weighing technic) of the apparatus or defective absorption by the chemical reagents. In the sampling method errors of this type persist, and there is in addition a series of errors introduced due to (*a*) incorrect measurement of the volume of expired air—whether dependent upon faults of the gas meter or failure to express the bag, or again to inaccurate measurement of temperature and barometric pressure; and (*b*) failures of technic in transferring samples to the gas analyser whereby, in particular, contamination with room air may be produced. With respect to errors of class (*a*), in our earlier experiments the measurement of temperature was imperfect because the thermometer was not inserted into the meter's inlet, and it was found, on subsequent investigation, that the temperature of the expired air at the moment of its volumetric measurement was understated to the extent of about 2° C.; this error was subsequently corrected. So far as the volume of expired air is concerned, careful experiment showed that with the light bag used in most of the experiments any error due to incomplete expression of the contents through the meter was very small. The combined effect of error classified under (*b*) and those inherent in any method of gas analysis was certainly more serious. We have attempted to appraise roughly the magnitude of such error in the following way. It is well known that if we have a series of paired readings of a magnitude, then the square root of half the mean square of the differences between the paired readings is a suitable measure of the error of observation, provided there be no correlation between the errors of paired determinations or between the magnitude of the error and that of the quantity measured. The former assumption cannot be strictly accurate because of secular change, that is to say, the more experienced the worker the smaller his error, so that some positive correlation surely exists between errors in each member of a pair, both will be smaller after some months' work than at the beginning of the research; the assumption would probably be quite correct if the analyst had had years of experience before the trial period. The second assumption was tested by correlating the difference between members of a pair with the mean value of the pair and it was found that the correlation in the series tested was not significant with respect to its error of sampling. The first assumption, despite its unsound theoretical basis, had to be retained owing to the impossibility of estimating satisfactorily the extent of the correlation mentioned. In the case before us we have two variables, viz., the CO_2 readings and the O_2 readings, and the error in the final estimation of calories expended is a function of the errors in each reading and the error correlation between CO_2 and O_2 readings (a correlation which will be in general negative for if, for instance, between the analyses a leak has occurred, which might easily happen, the CO_2 reading will go down and the O_2 reading go up). The square roots of the mean square errors (calculated on the above assumptions) and the correlation in error of oxygen and carbonic acid measurements were estimated twice, in the first case upon 23 paired analyses including results of both C. H. and A. E. T., in the second case upon 15 pairs in which both analyses were the work of C. H. Since the majority of the analyses used for calculation were due to C. H., more weight was assigned to the latter series which did not, however, differ very greatly from the former except in giving a lower value for the standard deviations of the oxygen errors and a somewhat lower value of the negative correlation between errors of CO_2 and errors of O_2 estimates. We reproduce the second set of data (Table I). From these errors, the errors of all the subsequently computed averages (respiratory quotient, O_2 use, etc.) can be deduced by algebraical reductions, and we finally reached the result that the error of estimate for the important constant, viz., the calorie use per minute, was of the order of 2 per cent. This result is in good accord with the estimate of C. H.'s working error reached by a quite different route. Two samples of

Table I.

Paired analyses (C. H.).			
CO_2 values.		O_2 values.	
4·32	4·23	16·22	16·47
4·40	4·28	16·03	16·18
3·78	3·29	16·68	16·65
3·78	3·28	16·68	16·75
3·29	3·28	16·65	16·75
4·13	4·12	16·51	16·62
4·13	4·08	16·51	16·58
4·12	4·08	16·62	16·58
3·96	3·91	16·30	16·37
2·87	2·78	17·68	17·65
3·31	3·23	17·46	17·51
3·13	3·07	17·98	18·00
4·48	4·45	16·38	16·44
3·09	2·96	17·21	17·27
3·67	3·67	17·02	17·07

expired air were analysed by C. H. using a small analyser, and by M. S. P., a much more experienced gas analyst, using the larger model. In one experiment, C. H.'s reading was 98·7 per cent. of M. S. Ps'., in the other 100·2 per cent. of his value (expressed as calories per unit). It would therefore seem that differences between two results reached by C. H. exceeding, say, 6 per cent. are unlikely to be attributable to errors of technic. It must, however, clearly be understood that we do not regard this estimate as more than a rude approximation. Quite apart from the doubtful truth of the theoretical assumptions, it is plain that the deduction of constants from so short and irregular a series as the one quoted here is hazardous (the deduced values are of course themselves subject to large errors of sampling), and, for that reason, we shall not reproduce the necessarily intricate arithmetical calculations requisite to determine the errors of the various constants. When two or three hundred paired analyses are available for statistical treatment it will be possible to import some degree of precision into this branch of the research. Had our enquiry been continued (as would have been the case if the war had not, fortunately, come to an end) we should have discussed the subject at length. We now pass to a still more difficult topic, viz., the interpretation of differences between experimental findings which lie outside the range which we have provisionally adopted as a margin of purely experimental error.

It is recognised that the likelihood that the difference between the mean values of two series of variable quantities has been produced by the operation of "chance" can be measured by calculating the respective "probable errors" of the averages. Not to pause upon the fact that the validity of this process in its ordinary application depends upon the assumption that the factors of chance variation are numerous, small, and result in a particular type of dispersion, we must recollect that in the present case we have special difficulties. If, for instance, we find that, judged by this criterion, the average energy expenditure in the press-house exceeds that in a lathe operation, to what extent is the difference contributed to by (*a*) a real difference of physiological demand made by the two kinds of work; (*b*) personal differences in the energy output of the workers themselves under all circumstances, functions of age, or temperament; (*c*) gross irregularities due to our having chanced upon keen workers in one operation and slack workers in another. It would have been possible to appraise (*b*) had we been able to measure the basal metabolism of our subjects, few though these were, but this we could not do owing to the conditions under which the research was done. If our numbers had been multiplied tenfold, it would have been fair to assume that differences under (*c*) were smoothed and equally distributed through the categories. But, things being as they were, the research being brought to an end when only a few hundred instead of a few thousand experiments had been made, we have no right to assume that (*a*) alone has been responsible for such "significant" differences as are to be discerned. The gross number of observations is so small that statistical manipulations and sub-tabulations designed to isolate the several factors (in addition to those above enumerated, are considerable variations of external temperature and cooling power) can only lead to a spurious air of precision. We have accordingly contented ourselves with a summary analysis, now to be detailed. The complete protocol of each experiment has been deposited with the Metabolism Research Sub-Committee, and the detailed particulars will be of use when sufficient material has been accumulated by other investigators to admit of a numerically adequate tabulation. The time for this is far distant, and the industrial aspects of metabolism cannot yet be fully elucidated.

Section 4.

In Tables II–XIII we record the numbers of experiments in each operation, the mean liberations of calories per minute and per square metre of body surface, the standard deviation, the percentage ratio of the standard deviation to the mean (coefficient of variation), and the approximate probable error of the mean (taken as 0·67449 multiplied by the standard deviation, divided by the square root of the number of experiments). Each

Table also records the individual experiments from which the general mean was computed, and the mean value for the series of experiments upon each subject. The anthropometric details relative to each person are set out in Table XIV.

Table II.

Light Turning.

Number of experiments	37
Mean calories per square metre and minute	1·596
Standard deviation	0·4356
Coefficient of variation	27·3
Probable error of mean ±	0·048

Mean temperature 7·3, kata—dry 10·2, wet 24·0.

Individual Series.

Ref. No.	Experiments.	——	Means.
21	3	1·21, 1·44, 1·42	1·36
22	4	1·20, 2·58, 1·65, 2·26	1·92
22A	10	1·46, 1·88, 1·57, 1·38, 1·93, 1·46, 1·15, 1·99, 1·82, 1·22	1·59
23	9	1·10, 1·00, 1·37, 0·98, 1·27, 1·99, 1·53, 1·51, 1·27	1·34
24	5	1·32, 1·83, 1·61, 1·32, 1·76	1·57
25	2	2·76, 2·70	2·73
26	1	1·55	1·55
29	3	1·38, 1·88, 1·32	1·53

Table III.

Turning and Finishing.

Number of experiments	36
Mean calories per square metre and minute	1·864
Standard deviation	0·5991
Coefficient of variation	32·1
Probable error of mean ±	0·067

Mean temperature 13·2, kata—dry 8·8, wet 21·7.

Individual Series.

Ref. No.	Experiments.	——	Means.
5	2	0·71, 1·38	1·05
6	3	1·37, 1·53, 2·29	1·73
7	3	1·11, 1·78, 1·40	1·43
8	4	1·71, 0·82, 0·87, 1·32	1·18
9	2	1·46, 2·32	1·89
10	6	2·93, 2·91, 3·11, 2·95, 2·53, 2·88	2·89
11	8	2·13, 2·11, 1·88, 1·66, 2·33, 2·03, 1·87, 1·81	1·98
12	8	1·56, 1·85, 1·76, 1·53, 1·50, 1·76, 2·00, 1·95	1·74

Table IV.

Tool Setting.

Number of experiments	25
Mean calories per square metre and minute	2·064
Standard deviation	0·6619
Coefficient of variation	32·1
Probable error of mean ±	0·089

Mean temperature 12·8, kata—dry 8·8, wet 22·2.

Individual Series.

Ref. No.	Experiments.	——	Means.
33	1	1·37	1·37
34	9	1·66, 1·83, 2·02, 2·04, 3·25, 1·23, 3·36, 1·91, 1·27	2·06
35	5	1·56, 3·09, 2·92, 1·60, 1·75	2·18
36	5	1·67, 1·40, 1·81, 2·64, 1·77	1·86
37	5	2·87, 2·82, 2·63, 1·15, 1·99	2·29

Table V.

Heavy Turning.

Number of experiments	21
Mean calories per square metre and minute	2·050
Standard deviation	0·4577
Coefficient of variation	22·3
Probable error of mean ±	0·067

Mean temperature 11·3, kata—dry 9·3, wet 23·1.

Individual Series.

Ref. No.	Experiments.	——	Means.
27	5	2·09, 1·70, 1·84, 2·21, 1·87	1·94
29	2	1·75, 2·22	1·99
30	8	1·84, 1·99, 2·24, 2·49, 2·45, 3·10, 2·87, 2·49	2·43
31	3	1·65, 1·48, 1·37	1·50
32	3	1·36, 2·36, 1·69	1·80

Table VI.

Forgings.

Number of experiments	20
Mean calories per square metre and minute	1·893
Standard deviation	0·3858
Co-efficient of variation	20·4
Probable error of mean ±	0·058

Mean temperature 13·1, kata—dry 9·9, wet 25·7.

Individual Series.

Ref. No.	Experiments.	——	Means.
1	3	1·62, 2·17, 1·76	1·85
2	7	1·35, 1·44, 1·86, 2·10, 1·40, 1·68, 1·70	1·65
3	7	2·11, 1·65, 2·68, 2·49, 1·97, 2·54, 1·50	2·13
4	3	1·60, 2·33, 1·90	1·94

Table VII.

Cleaning and Drying.

Number of experiments	20
Mean calories per square metre and minute	3·039
Standard deviation	0·8057
Coefficient of variation	26·5
Probable error of mean ±	0·122

Mean temperature 17·1, kata—dry 7·2, wet 19·7.

Individual Series.

Ref. No.	Experiments.	——	Means.
*16	4	2·74, 2·33, 2·30, 2·51	2·47
38	6	2·72, 3·09, 4·46, 4·97, 4·35, 4·21	3·97
39	1	2·84	2·84
40	4	2·58, 2·74, 3·25, 2·38	2·74
41	5	1·83, 2·52, 3·03, 2·79, 3·14	2·66

* C. H.

Table VIII.

Gauging.

Number of experiments	19
Mean calories per square metre and minute	2·567
Standard deviation	0·7071
Coefficient of variation	27·5
Probable error of mean ±	0·109

Mean temperature 11·7, kata—dry 8·8, wet 21·6.

Individual Series.

Ref. No.	Experiments.	——	Means.
*16	10	2·82, 2·75, 3·12, 3·02, 3·79, 2·86, 2·99, 2·27, 1·82, 3·05	2·85
19	4	2·56, 2·20, 3·92, 1·43	2·53
20	1	2·22	2·22
43	4	1·70, 2·98, 1·30, 1·98	1·99

* C. H.

Table IX.

Labouring.

Number of experiments	14
Mean calories per square metre and minute	3·069
Standard deviation	0·6456
Coefficient of variation	21·0
Probable error of mean ±	0·116

Mean temperature 14·3, kata—dry 8·2, wet 20·7.

Individual Series.

Ref. No.	Experiments.	——	Means.
13	4	3·65, 2·85, 3·67, 3·60	3·44
14	3	2·34, 3·33, 3·40	3·02
15	3	2·58, 2·09, 2·34	2·34
*16	3	2·07, 3·82, 3·94	3·28
17	1	3·29	3·29

* C. H.

Table X.

Stamping.

Number of experiments	12
Mean calories per square metre and minute	2·118
Standard deviation	0·3797
Coefficient of variation	17·9
Probable error of mean ±	0·074

No details of temperature or kata readings.

Individual Series.

Ref. No.	Experiments.	—	Means.
*16	7	1·89, 1·74, 1·86, 1·91, 1·94, 2·34, 2·00	1·95
17	5	2·22, 3·18, 2·24, 1·76, 2·34	2·35

* C. H.

Table XI.

Walking and Carrying.

Number of experiments	11
Mean calories per square metre and minute	2·688
Standard deviation	0·4729
Coefficient of variation	17·6
Probable error of mean ±	0·096

Mean temperature 10·5, kata—dry 9·5, wet 22·8.

Individual Series.

Ref. No.	Experiments.	—	Means.
*16	9	2·94, 2·93, 3·13, 2·35, 2·46, 3·46, 2·24, 2·79, 3·20	2·83
22A	1	2·20	2·20
29	1	1·87	1·87

* C. H.

Table XII.

Finishing Copper Bands.

Number of experiments	6
Mean calories per square metre and minute	2·125
Standard deviation	0·1695
Coefficient of variation	8·0
Probable error of mean ±	0·047

No details of temperature or kata readings.

Individual Series.

Ref. No.	Experiments.	——	Means.
*16	3	2·34, 2·32, 1·92	2·19
18	3	2·20, 2·01, 1·96	2·06

* C. H.

Table XIII.

Hoisting Shell (with pulley).

Number of experiments	5
Mean calories per square metre and minute	2·150
Standard deviation	0·5291
Coefficient of variation	24·6
Probable error of mean ±	0·160

Mean temperature 11·8, kata—dry 9·4, wet 21·9.

Individual Series.

Ref. No.	Experiments.	——	Means.
42	5	2·06, 2·74, 2·47, 2·29, 1·19	2·15

Table XIV.—Anthropometry.

Reference No.	Ages.	Heights.	Weights.	Surface.
		cm.	kgrm.	sq. metre.
1	22	165·4	62·1	1·687
2	25	161·6	68·5	1·728
3	32	164·1	65·9	1·719
4	27	149·9	54·7	1·487
5	19	154·9	56·2	1·541
6	19	174·9	56·0	1·680
7	19	150·2	56·2	1·508
8	19	165·7	56·2	1·618
9	20	150·2	46·8	1·394
10	19	165·4	55·8	1·611
11	25	165·7	65·4	1·726
12	33	168·3	51·8	1·618
13	47	155·3	57·6	1·560
14	46	172·7	86·5	2·003
15	51	160·0	63·3	1·659
16	44	165·1	55·2	1·601
17	35	159·4	44·2	1·421
18	42	166·4	57·2	1·634
19	27	158·4	51·9	1·514
20	18	163·8	55·1	1·591
21	22	167·6	58·1	1·655
22	25	165·1	48·1	1·510
22A	27	163·2	77·2	1·832
23	24	167·6	55·8	1·627
24	20	159·4	51·3	1·513
25	22	157·5	56·0	1·557
26	18	160·0	54·2	1·522
27	44	169·2	57·7	1·661
29	33	166·4	61·9	1·691
30	33	158·8	52·6	1·525
31	23	165·1	57·6	1·631
32	26	156·8	49·0	1·467
33	21	161·9	45·0	1·448
34	25	160·0	55·3	1·567
35	26	161·9	53·5	1·558
36	26	167·3	55·5	1·621
37	23	162·6	59·3	1·633
38	18	159·7	54·0	1·550
39	18	163·8	56·2	1·605
40	39	166·4	66·2	1·740
41	34	152·4	61·9	1·587
42	54	156·8	56·0	1·552
43	20	154·9	53·0	1·503

In the brief summarising Table XV the means are again shown, being here expressed as calories per hour per square metre given to the nearest whole number.

Table XV.—Summary.

Operation.	No. of persons.	No. of experiments.	Mean calories per sq. metre and hour.	Standard deviation.	Coefficient of variation.	Probable error of means.
1. Light turning	8	37	96	26 ·1360	27 ·3	±2 ·900
2. Turning and finishing ...	8	36	112	35 ·9477	32 ·1	±4 ·041
3. Tool setting	5	25	124	39 ·7137	32 ·1	±5 ·357
4. Heavy turning	5	21	123	27 ·4646	22 ·3	±4 ·043
5. Forging	4	20	114	23 ·1462	20 ·4	±3 ·491
6. Cleaning and drying......	5	20	182	48 ·3404	26 ·5	±7 ·291
7. Gauging......................	4	19	154	42 ·4246	27 ·5	±6 ·565
8. Labouring	5	14	184	38 ·7376	21 ·0	±6 ·983
9. Stamping	2	12	127	22 ·7827	17 ·9	±4 ·436
10. Walking and carrying...	3	11	161	28 ·3751	17 ·6	±5 ·771
11. Finishing copper bands	2	6	128	10 ·1691	8 ·0	±2 ·800
12. Shell hoisting (with pulley)	1	5	129	31 ·7468	24 ·6	±9 ·576

A consideration of the detailed Tables shows that, in a majority of operations, the range of variation from subject to subject is very great, in some cases the largest individual mean is as much as twice the size of the smallest. In some, for instance, tool setting, the subjects of several experiments come out fairly well together—the outlying value being based on but one experiment. In others the highest value is contributed by subject 16 (this is the case in gauging, walking, and finishing) who was not a trained operative. But no obvious extrinsic factor such as this will account for the general run of the results. Whether the extent of the variation is due to individual peculiarities of metabolism or to great variations in the amount of physical work actually performed by the various subjects during the several experimental periods, or whether both factors contributed, cannot be ascertained. We do not think anything of value would result from a numerical analysis of the data from this standpoint, and, *a fortiori*, we do not propose to relate the variations to those of temperature or cooling power. Both analyses must be undertaken when the supply of similar observations has been increased twenty or a hundredfold.

Provisionally, and with the greatest possible reserve, we should group the figures on the basis of the general means, bringing together those which, from the rough indications afforded by the probable errors, seem to fall into the same class.

Upon this basis it is seen that our subjects fall into four groups :—

First, we have light turning, turning, forging, which need in round numbers about 100 calories per square metre per hour.

The second group includes five operations, tool setting, heavy turning, stamping, finishing, copper bands, shell hoisting, all needing some 125 calories per square metre per hour.

The third group covers gauging and walking and carrying, to which 160 calories per square metre per hour may be allotted.

The fourth and last group contains labouring and cleaning and drying, which seem to need 180 calories per square metre per hour.

In using these provisional figures to assess the energetic requirements of the factory operative, we are to remember that the trend of public opinion points unmistakably to the conclusion that the industrial day will not for women exceed eight hours, and, if so, it would be improper to assume that more than a maximum of seven hours would be devoted to physical exertion approximating to that measured in our experiments. We accordingly think that the work contribution to the daily needs of an average woman in each of our classes will be reached if the above approximate values are multiplied by the product of 7 and 1·6, the latter being the larger of the two values for average body surface put forward in the Food (War) Committee's recent memorandum. Our four work quotas are accordingly

1120, 1400, 1792, 2016 calories.

In the memorandum just cited the allocation for needs outside working hours (including 240 calories for travelling and household work) amount to 1333 calories, to which we must add a further 77 calories, because the Committee envisaged 8 hours of actual industrial work, while we are contemplating only 7 hours. Hence, the quantity to be added to the work quota is 1410 calories, and the complete energy needs of our four classes are:—

2530, 2810, 3200, 3425 calories.

Or, making the customary allowance of 10 per cent. between net energy and food eaten,

2810, 3120, 3555, 3805 calories.

In comparison with the results deduced by the Committee from Becker and Hämäläinen's figures our highest class makes greater demands than any of theirs, our second highest figure being practically the same as that of the most strenuously employed women in the other series. While we are very far from thinking that our experiments are sufficient to provide a trustworthy figure, they are perhaps more representative of the requirements in a class of strenuously employed industrial women than those of Becker and Hämäläinen.

The unweighted mean of the food requirements is 3322 calories, and Dunluce and Greenwood, in their analysis of the dietaries of 19,213 munition workers (the large majority women), reached 3463 as the average man value.* Hence 2874 would be the value for an average woman. But this general summary includes some hostels for which the values were extremely low, and their largest homogeneous sample (about 7000 women in a great explosives supply factory) yielded a "man" value of 3951 calories and a "woman" value of 3279 substantially identical with our experimental approximation. Perhaps this concordance gives a somewhat greater credibility to our provisional conclusions. It is even possible that the 3279 represents a slight excess, because, if allowance is made for 1½ rest days (*vide infra*), our experimental figures of 3322 is reduced to 3039.

One important inference may be drawn from these figures. The energy requirement of the lightest class is about 74 per cent. of the heaviest. But the remuneration of the kinds of work included in our heaviest class is much less than that of several operations needing many fewer calories. If we supposed that so small a sum as 10*s*. would purchase an adequate weekly diet for a member of the lowest class, then assuming that the 1½ non-working days require the same allowance, which we may put at 1800 calories per diem, for all classes, the total weekly needs are 16,615 calories and 21,538 calories, so that the worker in the heavier class must spend nearly 13*s*. a week.

Section 5.

The issue raised at the end of the last section is of such importance that we may be permitted to discuss it in more detail than might seem fitting in a physiological report.

The data collected by the Board of Trade in 1904 showed that for families in receipt of less than 25*s*. weekly, 67·3 per cent. of total outgoings were expended upon food, the proportion only falling as low as 57 per cent. in families earning 40*s*. or more weekly. Even in the families of the poorest class studied by Rowntree, where the expenditure upon food was estimated to provide less than a maintenance diet, 51 per cent. of outgoings were for food.

The Working Classes Cost of Living Committee which reported in 1918 (Cd. 8980, 1918) provided data from which the following percentages have been calculated:—

---

* Medical Research Committee, Special Report, No. 13.

	Percentage of total expenditure devoted to the purchase of food.	
	1914.	1918.
Skilled workers	54·8	60·6
Semi-skilled workers	56·2	63·4
Unskilled workers	56·7	64·9

These figures refer to families composed of 4·57 "men." Let us now suppose that the average earning, viz., 75*s.* 5*d.* per week, is adequate to maintain the minimum standard of comfort in such a family, and that the principal wage earner is a "man" occupied as in our lightest class. Dividing the mean income by 4·57 and multiplying by 0·6 (on the assumption that the expenditure upon food is now about 60 per cent. of total outgoings), we find that the cost of food per "man" is almost exactly 10*s.* a week. If then the wage-earning "man" of a family similarly constituted in all other respects is in our heaviest physiological class, the general standard of living can only be maintained if the gross family income increased by nearly 3*s.* When it is remembered that the subsidiary earnings of other members of the family (which have always been relatively more important in the lower grades of manual labour) must be reduced by the new education proposals, the grave importance of taking into account the physiological element of working class expenditure is manifest. In any scientific appraisement of the income needed to maintain an agreed upon standard of life, it is essential to determine the minimum expenditure necessitated by the occupation of the wage earner. This investigation, fragmentary as it is, suffices to show that the variations of physiological demand when translated into terms of money are considerable. We cannot but express the opinion that the amount of attention which has been devoted to this aspect of the problem by economists and statesmen has been inadequate. Our inquiry has not been extensive enough to provide any basis for economic proposals, but does at least demonstrate the urgent need of further measurements.

In conclusion, we desire to express our sense of gratitude to the Ministry of Munitions for permission to carry out the work, to the manager and staff of the factory in which it was done, and to various scientific colleagues, in particular Prof. E. P. Cathcart, Dr. Leonard Hill, and Prof. E. H. Starling, for advice and criticism during its progress.

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The Effect of Nitrogen-fixing Organisms and Nucleic Acid Derivatives on Plant Growth.

By W. B. Bottomley, M.A., Ph.D.

(Communicated by Prof. F. W. Oliver, F.R.S. Received June 6, 1919.)

Introduction.

In a previous communication* it has been shown that a water extract of bacterised peat has a remarkable effect on the growth of *Lemna minor* in water-culture solution. The experiments there described showed that when supplied with inorganic nutrients only, the plants, though increasing in number, failed to maintain their normal size and health, and rapidly deteriorated, while the addition of a small quantity of the organic material resulted in a marked increase in the rate of multiplication, the plants at the same time showing improved health and vigour, proof of which was given by the increase in the dry weight of the plants so treated.

The author has also shown† that wheat seedlings, when deprived of their endosperm at a very early stage, will not grow normally unless supplied with organic matter. These experiments led to the conclusion that all plants, as well as animals, require a certain amount of *organic* substance for their proper development—a conclusion entirely contrary to the established view that plants can be grown in purely inorganic nutrients in water-culture.

In the course of the previous work, however, it was found that the organic matter is effective in extremely minute quantities, such as might conceivably be supplied by bacterial and algal contamination, though in these preliminary stages no attempt was made to determine the nature of the essential substances. The water extract of the bacterised peat, which was largely used, must be a complex mixture, containing constituents from both the peat and the bacteria, as well as the products of their interaction; and the growth-promoting effect may be due to any or all of these substances. The all-important bacteria used in the preparation of the bacterised peat are those concerned in nitrogen-fixation, and in May, 1917, a preliminary experiment was carried out with the object of ascertaining how far the effect of the bacterised peat extract was due to the products of these bacteria.

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* Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 89, pp. 481–507 (1917).

† Bottomley, W. B., 'Annals of Botany,' vol. 28, No. 111, pp. 531–540 (1914).

Effect of Azotobacter chroococcum.

Two series of five dishes each were arranged; the dishes in Series I, numbered from 1 to 5, each containing 250 c.c. of Detmer's solution, and those in Series II, numbered from 6 to 10, each containing 250 c.c. of the same solution, with the addition of 0·1 grm. of a growth of *Azotobacter chroococcum* in every 100 c.c. This growth was obtained by making streak cultures on agar-mannite plates from a pure colony of the organism, and incubating for about 14 days. At the end of this period a copious brown gelatinous growth was obtained over practically the whole surface, which was carefully scraped off with a spatula, transferred to a tared beaker, and rapidly weighed. The mass in the beaker was covered with distilled water, and the whole was raised to a temperature of 120° C. for one hour in an autoclave. The contents of the beaker were then measured and transferred to a well-stoppered bottle, the beaker and measuring cylinder being repeatedly rinsed out with measured quantities of distilled water, and the washings added to the bottle, until a volume of about 10 c.c. for every gramme of the gelatinous growth had been obtained. The stoppered bottle was placed in a shaking machine, and well shaken for six hours, at the end of which time the gelatinous mass appeared to be completely broken up and a uniform suspension obtained. A few drops of chloroform were added to this to ensure sterility.

When required for use, an aliquot portion of this liquid, containing the requisite quantity of bacterial growth, was measured out after the bottle had been well shaken, transferred to a beaker together with some distilled water and heated on a water-bath to a temperature of about 80° C. to remove the chloroform. When cold, the concentrated Detmer's solution and distilled water in sufficient quantity to make up the required volume were added. The Detmer's solution was prepared in quantity at one hundred times the normal concentration and kept in a stock bottle. These experiments were carried out entirely with conductivity water.

Ten similar plants of *Lemna minor* were counted out into each of the ten dishes, and 300 plants counted out at the same time for a determination of their dry weight. The dishes were covered with black paper to the surface of the liquid, as described in the previous communication, and the whole set placed in a greenhouse. The dishes were protected from dust by a large sheet of glass, supported at a height of about 2 in. above the top of the dishes. The culture solutions were changed twice weekly, and the plants counted once weekly. At the end of the third week the plants in Series II had almost filled their dishes, so the whole set was halved at the weekly counting, and this was repeated each week until the end of the experiment. When necessary the sets were even quartered, three-fourths of each dish being discarded. The figures obtained are shown in the Table below, the numbers given being the total numbers from the original ten plants in each dish, and not the fractions retained at the weekly countings.

Table I.

Series.	No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.
I. Detmer's Solution ...	1	22	80	212	576	1,248	1,984
	2	31	79	174	416	1,056	1,568
	3	30	82	166	464	1,024	1,696
	4	32	82	194	432	1,088	1,824
	5	35	86	210	512	1,248	1,824
Mean	—	32·0	81·8	191·2	480	1,132·8	1,779·2
II. Detmer's Solution + *Azotobacter*	6	40	135	532	2,624	10,240	25,120
	7	42	142	502	2,656	12,224	27,776
	8	37	130	564	2,720	10,656	27,872
	9	36	127	478	2,512	11,808	24,064
	10	43	159	600	2,848	11,040	28,832
Mean	—	39·6	138·6	535·2	2,672	11,193·6	26,732·8

At the end of the experiment the plants from three dishes in each set were used for a determination of the dry weight, and from the figures so obtained the dry weight of 100 plants was calculated. The results are shown below :—

	Mgrm.
Dry weight of 100 plants at beginning of experiment	14·8
„ „ from Series I at end of experiment ...	10·0
„ „ from Series II „ „ ...	18·2

It is evident from these figures that the addition of the sterilised *Azotobacter chroococcum* to the culture solution not only resulted in an increased rate of multiplication of the Lemna plants, but also enabled them to increase their original size, while the weight of the control plants showed a corresponding decrease. It therefore appears that part, at least, of the beneficial effect of the water extract of bacterised peat is due to the products of the nitrogen-fixing bacteria which it contains; and if such an effect as that recorded above be produced by a definite addition of bacterial substance, it is not unreasonable to suppose that a similar result might be attained in ordinary water-culture experiments by natural bacterial contamination, which is almost unavoidable unless the most scrupulous care be taken to ensure the sterility of the solutions and to change them frequently. It should be pointed out that the gelatinous growth of *Azotobacter* contained 96·68 per cent. of moisture, so that the addition of this material in the proportion of 1 grm. to 1,000 c.c. of solution represents only 33·2 parts of dry substance per million, containing 29 parts of organic matter. This quantity could quite conceivably be supplied by bacterial and algal contamination, and it is interesting in this connection to point out that it has been frequently observed that a more luxuriant growth is obtained in water-culture experiments when the solutions become contaminated with green algæ.

Effect of Nucleic Acid Derivatives.

It was not to be expected, however, that the whole of the effect of the bacterised peat extract was to be attributed to the products of these nitrogen-fixing bacteria; and since the effect of this organic extract was most marked upon the nuclei of the young developing plants, it was suggested that some nuclear constituent, present in the peat and rendered available during the process of "bacterisation," might be partially responsible for the results obtained.

It has already been shown* that on extracting raw peat with dilute alkalies and removing the "humic acids," by suitable means, certain nucleic acid derivatives can be obtained; and that by extracting the peat repeatedly with a 1 per cent. solution of sodium bicarbonate, these same nucleic acid derivatives are obtained, but the "humic acids" are not dissolved, so that the trouble of removing these latter substances is avoided. The nucleic acid derivatives thus obtained consist generally of an adenine-uracil dinucleotide and two mononucleotides—a guanine and a cytosine mononucleotide. The dinucleotide can be precipitated from the sodium bicarbonate extract, after concentration *in vacuo*, by an excess of absolute alcohol following the addition of sodium acetate and hydrochloric acid to the extract. A flocculent precipitate is obtained, which settles after about 24 hours to a fine powder, and this can be filtered off and washed with absolute alcohol. The two mononucleotides remain in the filtrate.

In order to test the effect of these various substances on the growth of *Lemna minor*, a weighed quantity of peat was repeatedly extracted with successive portions of a 1 per cent. solution of sodium bicarbonate until the extract was no longer coloured. The combined extracts, after carefully neutralising with hydrochloric acid, were concentrated *in vacuo* and divided into two equal portions, to one of which a few drops of chloroform were added, in order to prevent bacterial growth. This liquid was preserved in a bottle and known as "crude nucleic acid derivatives from raw peat." To the other half a little sodium acetate was added, and sufficient hydrochloric acid to render the liquid acid to litmus, and then about twice its volume of absolute alcohol to completely precipitate the dinucleotide. After 24 hours the liquid was decanted off through a filter, the precipitate washed with a little absolute alcohol, allowed to settle, and the liquid again decanted. This was repeated until the alcohol was no longer coloured. The precipitate was then dried *in vacuo* and dissolved up in a very slight excess of sodium carbonate solution, the excess being finally carefully neutralised with dilute hydrochloric acid. When made up to a known volume a few drops of chloroform were added, and the liquid, bottled for use, was known as "adenine-uracil dinucleotide from raw peat."

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* Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 90, pp. 39–44 (1917).

An experiment was then made in June, 1917, to test the effect on the growth of *Lemna minor* of each of these fractions from raw peat, in comparison with that of *Azotobacter chroococcum* and of bacterised peat. A set of thirty dishes was arranged in six series of five dishes each, and all contained 150 c.c. of Detmer's solution with the following additions:—Series I, numbered from 1 to 5, no addition; Series II, numbered from 6 to 10, the crude nucleic acid derivatives from 1 grm. of raw peat in every 500 c.c.; Series III, numbered from 11 to 15, the adenine-uracil dinucleotide from 1 grm. of raw peat per 500 c.c.; Series IV, numbered from 16 to 20, 0·5 grm. of the autoclaved growth of *Azotobacter chroococcum* per 500 c.c.; Series V, numbered from 21 to 25, the crude nucleic acid derivatives from 1 grm. of raw peat plus 0·5 gram of the growth of *Azotobacter chroococcum* per 500 c.c., *i.e.*, the addition made in Series II plus that made in Series IV; and Series VI, numbered from 25 to 30, the water extract of 1 grm. of bacterised peat per 500 c.c.

When the various extracts were required for use, the requisite quantities of the respective liquids were measured out from the stock bottles, transferred to evaporating basins, and warmed on the water-bath to a temperature of 80° C. to expel the chloroform. When cold they were added to the concentrated Detmer's solution and made up to the required volume with conductivity water.

Ten plants of *Lemna minor* were counted out into each dish, 300 similar plants being counted out at the same time for an estimation of their dry weight. The dishes were covered with paper to the level of the liquid, as described above, to cut out the light from the bottom and sides, and placed in a greenhouse as before. The solutions were changed twice weekly and the plants counted once weekly, and when they had filled their dishes in some of the series, as they had at the end of the second week in Series V and VI, the number throughout the set was halved at the weekly counting, only one-half of each dish being retained. This was repeated each week throughout the experiment, which lasted six weeks, the plants being even quartered when necessary. Table II shows the figures obtained, which represent the total numbers of plants arising from the original ten in each dish :—

Table II.

Series.	No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.
I. Detmer's Solution ...	1	29	84	256	352	704	1,472
	2	33	92	224	384	832	2,176
	3	32	96	208	384	704	1,536
	4	34	96	176	416	1,024	2,112
	5	33	88	208	384	768	1,792
Mean	—	30·2	91·2	214·4	384·0	806·4	1,817·6
II. Detmer's Solution + Crude Nucleic Acid Derivatives	6	44	212	900	3,008	16,896	109,824
	7	46	242	904	3,304	18,248	104,416
	8	39	238	836	3,160	16,568	84,528
	9	38	220	832	3,264	16,128	92,400
	10	47	244	864	2,880	14,848	93,440
Mean	—	42·8	231·2	867·2	3,123·2	16,537·6	96,921·6
III. Detmer's Solution + Dinucleotide	11	40	184	560	1,792	7,936	33,024
	12	41	196	640	1,984	7,168	30,208
	13	41	160	496	1,536	6,912	27,904
	14	36	172	560	1,728	8,448	35,584
	15	38	184	608	1,664	6,912	31,232
Mean	—	39·2	179·2	572·8	1,740·8	7,475·2	31,590·4
IV. Detmer's Solution + Autoclaved *Azotobacter*	16	40	168	592	2,048	12,288	69,376
	17	40	232	816	2,752	16,128	81,920
	18	45	208	784	3,072	18,432	91,136
	19	41	240	736	2,496	14,592	73,728
	20	42	212	784	2,944	17,664	84,736
Mean	—	41·6	212·0	742·4	2,662·4	15,820·8	80,179·2

Table II—*continued.*

Series.	No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.
V. Detmer's Solution + Crude Nucleic Acid Derivatives + *Azotobacter*	21	52	356	1,504	6,912	44,032	299,008
	22	50	344	1,680	7,936	40,448	277,504
	23	55	344	1,520	6,912	40,960	274,432
	24	45	320	1,472	7,232	44,544	323,072
	25	52	360	1,712	6,592	37,888	291,840
Mean	—	50·8	344·8	1,577·6	7,116·8	41,574·4	293,171·2
VI. Detmer's Solution + Bacterised Peat	26	50	324	1,536	9,088	47,104	311,808
	27	50	344	1,552	8,256	37,376	253,952
	28	55	372	1,856	8,128	41,472	302,592
	29	46	364	1,728	8,448	41,472	294,912
	30	50	336	1,296	7,552	38,400	301,056
Mean	—	50·2	348·0	1,593·6	8,294·4	41,164·8	292,864·0

From the second week onwards, when the plants were halved or quartered at the weekly countings, the discarded fractions from the five dishes in each series were added together and thoroughly washed, in order that an estimation of the dry weights of the plants could be made. From the data thus obtained a calculation was made of the dry weight of 100 plants in each series week by week, and a comparison of these weekly weights with the original weight of the plants is shown in the Table below :—

Table III.

Series No.	Weight of 100 plants in milligrams.					
	At beginning.	2nd week.	3rd week.	4th week.	5th week.	6th week.
I	16·4	16·7	11·0	9·6	4·9	4·7
II	16·4	19·3	18·7	20·1	18·1	18·6
III	16·4	19·3	18·4	17·9	18·8	17·7
IV	16·4	19·1	18·1	18·9	19·9	18·7
V	16·4	18·6	18·3	20·7	17·9	19·8
VI	16·4	19·6	18·6	17·1	20·0	19·7

The slight fluctuation shown in the weights of the plants in some series week by week, instead of a steady rise or fall, is explained by the irregular size of the small plants formed during the rapid multiplication of the large numbers obtained, resulting in a variable average weight for the series concerned.

It should be pointed out here that the increase in number of the plants in this experiment does not correspond with the figures given in Table I for a similar period. However, all experiments reported were carried out at different times, and each one is as complete as possible within itself, for it is impossible to make any correct comparisons between trials carried out at various periods of the year, owing to the great variation of such factors as duration of sunlight, temperature, etc.—factors which have a very great influence on the rate of growth and multiplication of the plants.

An examination of the numbers given in Table II shows that both the crude nucleic acid derivatives in Series II and the autoclaved *Azotobacter* in Series IV have the effect of markedly increasing the rate of growth and multiplication of the Lemna plants; and the figures in Table III show that the average weights of the individual plants in the same series increased beyond their original weight, while the weight of the plants in the control series steadily decreased. It is evident, therefore, that both the crude nucleic acid derivatives and the *Azotobacter* have a growth-promoting effect; but when the two are added together to the Detmer's solution, as in Series V, their combined effect is far greater than the sum of their effects when added separately, as in Series II and IV, and is approximately equal to that of the water extract of bacterised peat in Series VI. It would therefore appear that the growth-promoting substances in these two liquids are dissimilar in their action upon the plant, and that they are in some manner complementary to one another; for were they similar in their *rôle* in the plant metabolism, it would be expected that the effect of the two when supplied together would be approximately equal to the sum of their separate effects.

The remarkable similarity between the results produced by the extract from 1 grm. of bacterised peat, on the one hand, and the crude nucleic derivatives from 1 grm. of raw peat, together with the *Azotobacter* growth, on the other, is readily explained. It may reasonably be supposed that the water extract from 1 grm. of bacterised peat contains a quantity of the nucleic acid derivatives approximately equal to that in 1 grm. of raw peat, these substances having been rendered water-soluble during the process of "bacterisation"; while, since *Azotobacter chroococcum* is very largely used during this process, and multiplies rapidly in the peat basis, it is probable that the water extract of the material contains a quantity of the products of this organism comparable to the proportions used in the above experiment, since this proportion would amount to only 1·68 per cent. of the weight of the peat.

Effect of Bacillus radicicola.

A further experiment was started in September, 1917, to test the effect of the nitrogen-fixing organism of leguminous nodules—*Bacillus radicicola*—and to compare it with that of *Azotobacter chroococcum.* The rate of growth and multiplication was necessarily slow in this advanced season of the year, and therefore the margin for experimental error became greater, but in spite of these objections fairly uniform results were obtained.

Fifteen dishes were arranged in three series of five dishes each. All contained 150 c.c. of Detmer's solution, and the following additions were made:—Series II, 1 grm. of autoclaved *Azotobacter chroococcum* per 1000 c.c., and Series III, 1 grm. per 1000 c.c. of autoclaved *Bacillus radicicola*, grown in the same manner on maltose-agar plates.

Table IV.

Series.	No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.
I. Detmer's Solution	1	19	42	80	100	120	160	176	216
	2	21	50	104	140	144	160	200	224
	3	21	43	88	108	136	200	224	240
	4	20	42	92	124	168	184	192	240
	5	21	51	108	132	160	224	208	248
Mean	—	20·4	45·6	94·4	120·8	145·6	185·6	200·0	233·6
II. Detmer's Solution + *Azotobacter*	6	24	57	130	196	276	420	556	732
	7	22	57	116	176	232	404	548	672
	8	21	50	118	184	264	416	512	664
	9	20	50	110	172	232	392	500	664
	10	24	59	122	192	276	424	596	740
Mean	—	22·2	54·6	119·2	184·0	256·0	411·2	542·4	694·4
III. Detmer's Solution + *Bacillus radicicola*	11	22	50	124	196	296	484	676	852
	12	21	46	104	172	248	412	628	844
	13	22	58	128	196	280	424	616	872
	14	23	57	124	200	288	520	696	928
	15	26	59	128	200	280	464	624	832
Mean	—	22·8	54·0	121·6	192·8	278·4	460·8	648·0	865·6

Ten similar Lemna plants were counted out into each dish, and 300 also thoroughly washed for an estimation of their dry weight. The dishes were treated precisely as in the preceding experiments with regard to the exclusion of light from bottom and sides and protection from dust. The solutions were changed twice weekly, and the plants counted once weekly, one half of each dish being rejected at the weekly counting when necessary. The numbers obtained are shown in the Table IV.

Estimations of the dry weights of the fractions discarded at the weekly countings were made at the end of the third, fifth, and eighth weeks respectively, with the following results:—

Table V.

Series No.	At beginning.	3rd week.	5th week.	8th week.
I	10·9	10·8	9·9	6·5
II	10·9	12·2	16·0	18·5
III	10·9	12·5	15·9	19·1

These figures indicate that *Bacillus radicicola* is quite as effective as *Azotobacter chroococcum* in promoting the growth of Lemna plants.

Effect of the Ash Constituents.

In all the experiments hitherto described, it has been assumed that it is the *organic* constituents of the additions to the nutrient solutions which have brought about the large increases in growth. There was the possibility, however, that minute quantities of certain *inorganic* substances present in the materials might function as activators, and thus account for the results obtained. In order to test this, fresh quantities of the crude nucleic acid derivatives, and of the *Azotobacter* growth were prepared as above described, in May of 1918, and each was divided into two equal parts. One half of each was carefully evaporated to dryness in a porcelain dish over a water bath, and the residue completely incinerated. When cold the ash was ground to a fine powder with a little water, and carefully transferred to a calibrated stoppered flask, the dish being rinsed with successive small quantities of conductivity water, which were added to the flask. The whole was made up to a known volume, so that an aliquot portion of the well-mixed contents could be taken, representing the ash from a known weight of the original material used.

Twenty-five dishes were then arranged in five series of five dishes each, and all contained 150 c.c. of Detmer's solution. Series I, containing dishes numbered from 1 to 5, constituted the control series, and to the dishes of the other series the following additions were made: Series II, numbered from 6 to 10, the ash from the crude nucleic acid derivatives from 1 grm. of raw peat per 500 c.c. of solution; Series III, numbered from 11 to 15, the crude nucleic acid derivatives in a similar proportion; Series IV, numbered from 16 to 20, the ash from 1 grm. of *Azotobacter* growth per 1000 c.c.; and Series V, numbered from 21 to 25, 1 grm. of the original *Azotobacter* growth per 1000 c.c. Ten similar plants of *Lemna minor* were counted out into each of the dishes, which were covered on bottom and sides with black paper. Three hundred plants of size comparable with those used in the dishes were counted out at the same time for an estimation of their dry weight. The plants were grown under similar conditions to those recorded in the above experiments, solutions being changed twice weekly while the plants were counted once weekly, and halved or quartered when necessary. The following are the results obtained:—

Table VI.

Series No.	Dish No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.
I. Detmer's Solution	1	37	86	252	464	768	1,344	3,776
	2	34	79	252	480	768	1,408	4,416
	3	34	72	232	496	896	1,536	4,800
	4	34	77	220	480	992	1,536	4,992
	5	33	76	264	464	800	1,408	4,224
Mean	—	34·4	78·0	244·0	476·8	844·8	1,446·4	4,441·6
II. Detmer's Solution + Ash from Nucleic Acid Derivatives	6	32	73	216	472	864	1,536	4,928
	7	46	85	252	528	896	1,408	4,288
	8	46	86	264	448	960	1,536	5,504
	9	40	94	244	528	768	1,472	4,544
	10	35	83	220	464	768	1,408	3,840
Mean	—	39·8	84·1	239·2	488·0	851·2	1,472	4,620·8
III. Detmer's Solution + Nucleic Acid Derivatives	11	33	128	436	1,272	3,136	9,472	34,688
	12	52	132	480	1,128	3,360	8,960	38,144
	13	41	127	432	1,184	3,584	10,176	39.232
	14	42	113	468	1,224	3,424	9,984	39,680
	15	39	117	412	1,208	3,008	9,024	38,912
Mean	—	41·4	123·4	445·6	1,203·2	3,302·4	9,523·2	38,131·2

Table VI—*continued.*

Series No.	Dish No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.
IV. Detmer's Solution + Ash from *Azotobacter*	16	37	93	244	480	864	1,344	3,392
	17	36	86	240	536	800	1,280	4,096
	18	37	83	244	472	864	1,472	4,480
	19	37	96	264	480	960	1,472	4,160
	20	39	78	240	488	928	1,472	4,608
Mean	—	37·2	87·2	246·4	491·2	883·2	1,408·0	4,147·2
V. Detmer's Solution + *Azotobacter*	21	40	99	340	872	1,632	4,864	25,216
	22	36	102	340	832	2,048	5,184	26,048
	23	47	102	324	776	1,760	4,928	22,464
	24	48	108	328	800	1,792	4,480	25,024
	25	43	108	324	792	2,048	5,568	28,608
Mean	—	42·8	103·8	331·2	814·4	1,856·0	5,004·8	25,472

The weights of the fractions discarded week by week, as compared with the original weight of the plants, is shown in the following Table:—

Table VII.

Series No.	Weight of 100 plants in milligrams.					
	At beginning.	3rd week.	4th week.	5th week.	6th week.	7th week.
I	14·6	11·2	11·0	10·4	9·6	8·9
II	14·6	11·7	11·3	10·9	10·5	9·4
III	14·6	14·2	15·2	20·6	20·9	20·5
IV	14·6	11·5	10·3	9·7	9·1	8·1
V	14·6	14·3	14·9	18·6	18·8	18·3

The ash of the nucleic acid derivatives and the *Azotobacter* had evidently not the slightest effect on the growth of *Lemna minor*, and the beneficial results following the addition of these materials can only be attributed to their organic constituents.

Conclusion.

The chief interest of the present work centres around two facts: first, that the addition of the derivatives from nucleic acid which has undergone decomposition in decaying vegetable matter—peat—has a marked effect on the growth of Lemna plants in water culture; and second, that the nitrogen-fixing bacteria have the power of elaborating products, which also greatly increase the growth of the Lemna plants.

All of these organic materials—the crude nucleic acid derivatives and the *Azotobacter* and *Bacillus radicicola* growths—give the Folin-Macullum[*] reaction, which is stated to indicate the presence of the growth accessory substances—vitamines—which are so important in animal nutrition.

In connection with the effect of the nucleic acid derivatives it is interesting to note that the pure adenine-uracil fraction has not the same effect as the crude extract containing all the products of decomposition. This is in accordance with the work of Schreiner and Skinner,[†] who found that pure nucleic acid increased the growth of plants in water culture, while some of its derivatives, as hypoxanthine and guanine, though still increasing growth, were not nearly so effective.

Whatever may be the nature of these growth-promoting substances, it is a noteworthy fact that they can be synthesised by the nitrogen-fixing bacteria from a carbohydrate and elementary nitrogen. It remains to be seen whether the products of other bacteria will bring about the same effect, but in working with organisms other than those which fix nitrogen, one great drawback is that a start has to be made by supplying a nitrogenous food material, so that in the bacterial growth there are present not only nitrogenous substances elaborated by the organisms, but also degradation products of this food substance.

It may be that among the products of the nitrogen-fixing bacteria are to be found substances similar in nature to those contained in the crude nucleic acid derivatives from raw peat, and an examination of these products is now in progress to determine whether such substances do occur. In any case, the experiments recorded throw an additional light on the possible *rôle* of the nitrogen-fixing bacteria in the soil, especially in view of the generally accepted fact that the presence of these organisms is always an indication of soil fertility.

The greater part of this work was carried out in 1917, but owing to the author's illness early in 1918 he was unable to complete and publish it until the present time. He wishes to express his sincere thanks to Miss Mockeridge, D.Sc., for her invaluable assistance in the work.

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[*] Folin and Macullum, 'Journ. Biol. Chem.,' vol. 11, p. 265 (1912).

[†] Schreiner and Skinner, 'U.S. Dept. of Agric., Bureau of Soils,' Bull. No. 87.

The Vegetative Morphology of Pistia *and the Lemnaceæ.*

By Agnes Arber, D.Sc., F.L.S., Fellow of Newnham College, Cambridge.

(Communicated by Prof. F. W. Oliver, F.R.S. Received June 17, 1919.)

Introduction.

The nature of the duckweed "frond" has presented a baffling problem to botanists since the early days of vegetable morphology. A detailed history of the views that have been held on the question need scarcely be attempted here, since the extreme reduction of the Lemnaceæ has offered scope for wild surmises of little scientific value.* The principal theories which have been put forward may, however, be summarised as follows:—

The "fronds" of the Lemnaceæ have been regarded as:—

(1) Entirely axial (Hegelmaier, F. (1868)).

(2) Entirely foliar (Goebel, K. (1891–3)).

(3) Foliar in the distal region and axial in the proximal region (Horen, F. van (1869), Engler, A. (1877), and Velenovský, J. (1907)).

The objections to (1) and (2) are obvious: these views can only be maintained if—in the first case—certain essentially foliar qualities be attributed to stem organs, and—in the second case—if leaves be assumed to possess some of the distinctive properties of stems. It is not inconceivable that such assumptions might find justification, but they should only be used as a last resort, in the case of no adequate explanation on normal lines being forthcoming.

The third view, first suggested by van Horen,† has been placed on a thoroughly sound basis by Engler's‡ exhaustive comparative study of the morphology of the more typical Araceæ and of their relation to *Pistia* and the Lemnaceæ. There is no doubt that the comparison of the river lettuce (*Pistia Stratiotes*, L.) and the duckweeds supplies the clue to the problem. Engler interprets the region of the frond of the Lemnaceæ above the basal "pockets" (Taschen) as of foliar nature, and he considers the buds developed in these pockets as the equivalents of the lateral shoots of *Pistia*, the main difference being that, in the river lettuce, only one bud is developed in connection with each leaf, while in the duckweeds there are two, one on each side. The position of the buds, which are lateral in relation to the leaf-limb, is the same in both. In the Lemnaceæ the axis of the vegetative plant is so far reduced from the *Pistia* type as to bear only one leaf in a pseudo-terminal position, with two lateral buds; while in *Pistia* the stem bears a rosette of numerous closely-placed leaves accompanied by a series of lateral shoots.

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* See, for example, Dutailly, G. (1878).

† Horen, F. van (1869).

‡ Engler, A. (1877).

The soundness of Engler's general position in regard to *Pistia* and the Lemnaceæ can scarcely be doubted by any botanist who studies his memoir on the Araceæ. There remain, however, two questions, to which he does not allude, but which seem to me to require answers. These questions are:—

(1) What region of the leaf do the "limb" of *Pistia* and the distal part of the "frond" of the Lemnaceæ represent?

(2) What is the exact morphological nature of the "pockets" of the Lemnaceæ, and what is their equivalent—if any—in the *Pistia* shoot?

The present paper is an attempt to find some solution of these two problems.

The Vegetative Morphology of Pistia Stratiotes, *L.*

The main part of the leaf of *Pistia* consists of a limb which is ob-cuneate or fan-shaped, with parallel veins, deeply grooved, and, especially in young stages, densely hairy. It is often much swollen—particularly in the median basal region—with air-containing tissue. A ligule, which is sheathing in its upper portion, occurs between the "limb" and the axis at the extreme base of the former.* This makes it obvious that the "limb" cannot be interpreted as of the nature of a leaf-sheath, and we are left with the alternatives that it may represent petiole, or lamina, or both. On the phyllode theory of the Monocotyledonous leaf, which I have advocated in a previous paper,† we should expect the lamina to be absent, while the limb would be of the nature of a flattened petiole—and there is nothing in its form and venation to make this view untenable. But for positive evidence we must look to the internal structure. Attention was drawn some years ago by a Japanese botanist‡ to certain features of the leaf anatomy, but the peculiar arrangement of the vascular bundles appears to have been hitherto overlooked. I have found, on cutting transverse sections, that, in addition to more than one row of normally orientated strands (*n.b.*, in figs. 1 and 2), there is, towards the upper surface of the leaf, a series of bundles with inverted orientation, *i. b.* In the paper already cited I have given reasons for regarding the presence in a leaf of such inverted bundles as an indication of petiolar origin. We may thus conclude that the relation of the leaf-limb of *Pistia Stratiotes* to its ligule, together with the evidence of its form, venation, and vascular anatomy, appear to

* Domin, K. (1911).
† Arber, A. (1918).
‡ Ito, T. (1899).

indicate that this organ is of the nature of a petiolar phyllode, flattened in the horizontal plane.

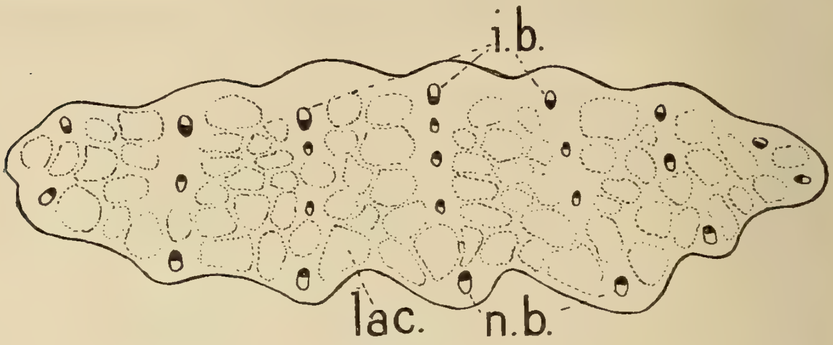


Fig. 1.

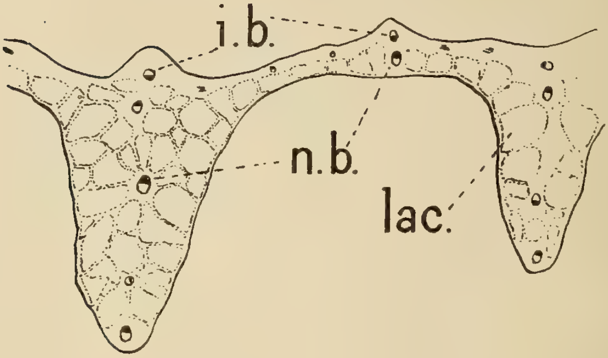


Fig. 2.

Figs. 1 and 2.—*Pistia Stratiotes*, L. Transverse sections of leaf (×21). Fig. 1, complete section near base of leaf. Fig. 2, two of the ribs in the median region of the fan-shaped limb; *n.b.*, normally orientated vascular bundle; *i.b.*, inversely orientated bundle; *lac.*, lacuna.

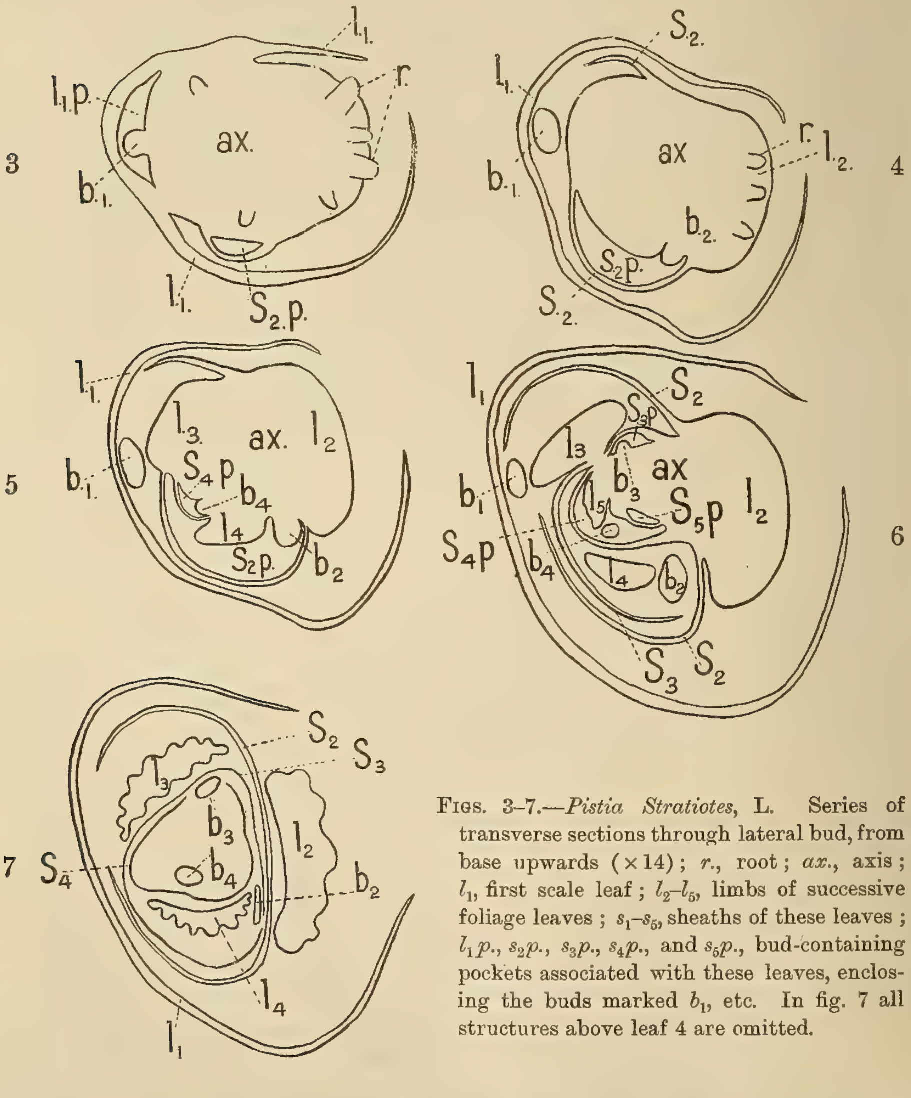
The distal region of the frond of *Spirodela polyrrhiza*, Schleid., the greater duckweed, bears a strong general resemblance to that of *Pistia*, both in form, venation, and development of air-tissue. The vascular system is, however, much simplified, and only one series of bundles remains. These are normally orientated, but in such a reduced type of leaf, in which, as compared with *Pistia*, several rows of bundles may be supposed to have been lost, it is not surprising that the inversely orientated series has failed to survive. There seems every reason to interpret the distal part of the frond of the Lemnaceæ as a petiolar phyllode—exactly equivalent to that of *Pistia*—especially if, as we hope to show, both sheath and ligule of the river lettuce are represented in the proximal region of the frond of the greater duckweed.

When we turn to Engler's figures and description of the *Pistia* shoot, we find no indication of anything corresponding to the "pockets" of the Lemnaceæ, while the buds are represented as situated *between* the ligule and the limb of the leaf. No drawings are given showing the actual origin of the buds from the axis, the diagrammatic transverse sections of shoots being all taken above the level of the apex of the axis. Engler's paper was published in the seventies of the last century, that is to say before the microtome had become one of the recognised instruments of botanical research. Now it happens that the extreme abbreviation of the axis in *Pistia* makes it almost impossible to follow the detailed relations of the parts of the shoot without the help of serial sections; this difficulty is enhanced by the fact that the levels at which the leaves become free from the axis are not consistent with their actual order of development (*cf.* figs. 3–7). It is probably for these reasons that my re-examination of *Pistia* has led to somewhat different results from those of Engler—results which serve, however, to corroborate and extend his general view regarding the relation of *Pistia* to the Lemnaceæ.

It is not easy in this country to obtain supplies of *Pistia* plants with actively growing axillary shoots suitable for microtoming, and the present account is based upon material in which only two buds proved perfectly adapted for the purpose. Fortunately one of these, which was entirely vegetative, displayed the general structure with great clearness. I am indebted to the Director of the Royal Botanic Gardens, Kew, for living plants, and to Prof. A. C. Seward, F.R.S., for herbarium material.

The axis of the developing bud of *Pistia*, which is illustrated by the transverse sections shown in figs. 3–7, bore, firstly, a zone of adventitious roots, r, in fig. 3, immediately followed by a sheathing scale-leaf, l_1. This was not attached to the axis in the median line, but considerably to one side, thus showing an asymmetry which was also displayed by the sheathing region of the normal leaves. The broader free segment was again fused with the axis at a second point of the circumference, thus forming a small pocket ($l_1p.$). Within this pocket a bud, b_1, was developed from the axis. The second leaf, l_2, which was a normal foliage leaf, behaved in an essentially similar manner, with certain modifications due to the possession of a differentiated limb. Below the level at which the limb became free from the axis, there was a distinct sheath forming a free flap, s_2, on one side, while on the other side it was free for a short distance laterally and then again attached to the axis, thus forming a pocket ($s_2p.$) containing a bud, b_2. Higher up, the margin of the pocket became free like the opposite sheath-wing (*cf.* figs. 5 and 6), and, higher still, this sheath detached itself entirely from the limb, and the two wings fused into a free axillary ligule (fig. 7).

The bud permanently occupies a position at the side of the limb, and lies between the sheath of the leaf to which it belongs and the axis. It is not necessary to follow the history of the succeeding leaves, l_3, l_4, etc., which form bud-pockets in a precisely similar way. The peculiar lateral position of the buds may perhaps be interpreted as due to the congenital fusion of the base of the limb with the axis; this fusion obliterates the space which would normally be occupied by the bud.


3
l.1.
l.1.p.
r.
ax.
b.1.
l.1.
S2.p.
4
S.2.
l.1.
r.
l.2.
ax
b.1.
b.2.
S2p.
S.2.
5
l.1.
l.3.
ax.
l2
b.1.
S4 p
b4
l4
S2p.
b2
6
l1
S2
S3p
l3
b3
ax
b1
l5
S5p
l2
S4p
b4
l4
b2
S3
S2
7
S2
S3
l3
b3
l2
S4
b4
b2
l4
l1

FIGS. 3–7.—*Pistia Stratiotes*, L. Series of transverse sections through lateral bud, from base upwards (×14); *r.*, root; *ax.*, axis; l_1, first scale leaf; l_2–l_5, limbs of successive foliage leaves; s_1–s_5, sheaths of these leaves; $l_1p.$, $s_2p.$, $s_3p.$, $s_4p.$, and $s_5p.$, bud-containing pockets associated with these leaves, enclosing the buds marked b_1, etc. In fig. 7 all structures above leaf 4 are omitted.

It will be recognised, from the description just given and from the accompanying figures, that my observations conflict with those of Engler, who regards the bud as occupying an anomalous position *outside the sheath.* I am also unable to confirm Velenovskýs* statement that the buds are originally median, but are forced by pressure to occupy a lateral position; they are undoubtedly, from the first, completely lateral to the limb, though lying inside the sheath.

The Comparison of Pistia *and* Spirodela.

The interest of the points in which my results conflict with those of Engler and Velenovský, lies in the fact that, in every case in which I differ from these two writers, my results point to an even closer morphological relation between *Pistia* and the Lemnaceæ than has been hitherto claimed. It seems clear that the lateral bud-containing pockets formed by the sheath of *Pistia* are exactly equivalent to the pockets of the Lemnaceæ. The sketch of a *Spirodela* plant seen from below (fig. 8) will perhaps help to explain this relation. The petiolar phyllode, *l*, passes at its base into the reduced and flattened axis, *ax.*, of which it forms the apparent continuation, exactly as the spathe of *Acorus* continues down into the scape, but with the difference that in *Spirodela* the growing point of the axis completely aborts, whereas in the fertile shoot of *Acorus* it forms the spadix. Just where the limb of the duckweed frond fuses with the axis, a group of roots, *r.*, takes its origin. We find an analogy to this in *Pistia*, where fig. 4 shows roots arising from the base of the limb of leaf 2, at the level of its junction with the axis. The two wings of the sheath of *Spirodela*, *s* and *s′*, are developed, just as in *Pistia*, below the attachment of the limb to the axis. They do not meet and fuse into a

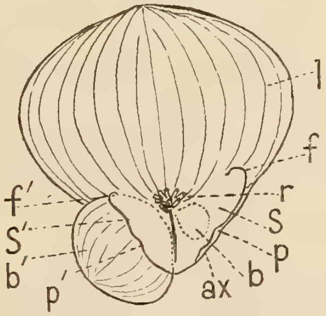


FIG. 8.—*Spirodela polyrrhiza*, Schleid. A plant viewed from the underside (enlarged); *l.*, limb of leaf belonging to main axis, *ax.*; *r.*, roots (cut short); *p.* and *p′.*, pockets between wings of sheath (*s.* and *s′.*) and axis, enclosing buds, *b.* and *b′.*; *f.* and *f′.*, ligular flaps of sheath.

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* Velenovský, J. (1907).

conspicuous axillary structure, as in *Pistia*, but the two small free flaps, *f* and *f′*, may be interpreted as reduced ligules. The basal regions of the two sheath-wings each form a bud-containing pocket, *p* and *p′*, equivalent to the one sheath-pocket accompanying each leaf in *Pistia*. These pockets are ultimately open in *Spirodela*, but in younger stages they are closed in the basal region as in *Pistia*. The development of the buds in pockets between the sheath and axis, below the limb and occupying a lateral position with regard to it, is thus identical in *Pistia* and the Lemnaceæ.

Summary.

Anatomical examination of the "limb" of the leaf of *Pistia Stratiotes*, L., the river lettuce, shows that, in addition to normally orientated vascular bundles, there is a series of inverted bundles towards the upper surface. This fact is regarded as indicating that the leaf is of the nature of a petiolar phyllode. This interpretation is extended to the distal part of the frond of the Lemnaceæ (duckweeds).

The general view, put forward by Engler 40 years ago, as to the morphological relation of the Araceæ—through *Pistia*—to the Lemnaceæ, is accepted in the present paper, and it is shown that more detailed investigation by modern methods makes it possible to carry the comparison considerably further. Serial sections through a developing shoot of *Pistia* reveal the presence of a "pocket" in connection with each leaf, occurring below the level of the free part of the limb; this pocket is formed on one side by the leaf-sheath, and on the other side by the axis, and encloses a bud occupying a lateral position in relation to the limb of the leaf. It is shown that these pockets are exactly equivalent to the pockets at the base of the frond in the Lemnaceæ, which, in the case of *Spirodela*, are easily seen to be formed, on the lower side, by the wings of the leaf-sheath, terminating in two minute ligular flaps, and, on the upper side, by the axis.

Acknowledgments.

I have to acknowledge a grant from the Dixon Fund of the University of London in aid of this and other researches carried out at the Balfour Laboratory, Cambridge.

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The Genesis of Œdema in Beriberi.

By Robert McCarrison, M.D., D.Sc., F.R.C.P., Brevet Lieutenant-Colonel, Indian Medical Service.

(Communicated by Prof. J. G. Adami, F.R.S.—Received June 19, 1919.)

In a previous communication (1) it was shown: (1) that in avian beriberi (polyneuritis gallinarum) œdema was constantly (100 per cent.) associated with massive enlargement of the adrenal glands; (2) that 82.2 per cent. of cases having such enlargement of the adrenal glands presented evidences of œdema in some form; (3) that the enlargement of the adrenals was associated with a corresponding increase in the adrenalin-content, as determined by physiological means. Additional evidence confirmatory of these findings is recorded in the present paper.

Avian beriberi was produced experimentally in a fourth series of 22 young pigeons by means of a dietary of autoclaved milled rice (2 and 3). Detailed *post-mortem* examinations were made in all cases. The heart's blood was examined by aërobic cultural methods for bacterial organisms. Ten of the 22 cases presented evidences of œdema (Tables II and III).

The adrenal glands were removed, weighed, and the adrenalin-content immediately estimated by the method of Folin, Cannon, and Dennis (4). Similar estimations were made of the adrenalin-content of the adrenals in

10 healthy control pigeons of a like age to those in which beriberi was experimentally produced.

In previous communications (1, 2) the weights of the adrenals per kilogram of body-weight were calculated against the original body-weights of the birds. In the present series of cases of avian beriberi the weights of these organs are shown in terms of (*a*) original body-weight, as well as in terms of (*b*) final body-weight of the birds at the time of death. The results of these observations are shown in the following Tables:—

Table I.—Showing the Adrenalin-Content in the Adrenal Glands and the Total Adrenalin per kilogram of Body-Weight in 10 Healthy Control Pigeons.

Number of Pigeon.	Final weight of pigeon in grm.	Weight of adrenals in mgrm.	Weight of adrenals per kilogram of body-weight in mgrm.	Total adrenalin in glands in mgrm.	Total adrenalin per kilogram of body-weight in mgrm.
242	350	30	85·7	0·090	0·257
255	230	22	95·6	0·051	0·221
256	210	24	114·2	0·054	0·257
257	220	22	100·0	0·052	0·238
258	240	18	75·0	0·045	0·187
259	280	22	78·5	0·052	0·187
260	240	22	91·6	0·051	0·212
261	270	24	88·8	0·054	0·200
262	270	23	85·0	0·060	0·222
263	240	25	104·0	0·045	0·187
Averages ...	255	23·2	91·8	0·0554	0·216

Analysis of Table I:—

(1) In healthy pigeons in this series, the weight of the adrenals per kilogram of body-weight ranged between 75 and 114 mgrm.

(2) The total amount of adrenalin in the healthy glands ranged between 0·045 and 0·090 mgrm.

(3) The average adrenalin-content per gramme of gland in ten *healthy* pigeons was 0·0023 grm.

(4) The total amount of adrenalin per kilogram of body-weight ranged between 0·187 and 0·257 mgrm.

Table II.—Showing the Adrenalin-Content in the Adrenal Glands and the Total Adrenalin per kilogram of Body-Weight in 12 Cases of Avian Dry Beriberi.

Number of pigeon.	Original weight of pigeon in grm.	Final weight of pigeon in grm.	Weight of adrenals in mgrm.	Weight of adrenals per kilogram of original body-weight in mgrm.	Weight of adrenals per kilogram of final body-weight in mgrm.	Total adrenalin in glands in mgrm.	Total adrenalin per kilogram of original body-weight in mgrm.	Total adrenalin per kilogram of final body-weight in mgrm.
238	240	170	25	104	147	0·045	0·187	0·264
223	230	140	29	126	207	0·045	0·195	0·321
222	270	210	40	148	190	0·084	0·311	0·400
228	245	170	21	85	123	0·048	0·195	0·282
243	250	210	62	248	295	0·138	0·552	0·657
241	230	160	33	143	206	0·057	0·247	0·356
237	260	150	41	157	273	0·090	0·346	0·600
250	190	155	27	142	174	0·075	0·394	0·483
226	250	190	31	124	163	0·090	0·360	0·473
246	200	130	55	275	423	0·105	0·525	0·807
230	250	180	37	148	205	0·090	0·360	0·562
224	260	160	30	115	187	0·045	0·173	0·250
Average ...	239	168	36	151	216	0·076	0·320	0·454

Table III.—Showing the Adrenalin-Content in the Adrenal Glands and the Total Adrenalin per kilogram of Body-Weight in 10 Cases of Avian Wet Beriberi.

Number of pigeon.	Original weight of pigeon in grm.	Final weight of pigeon in grm.	Weight of adrenals in mgrm.	Weight of adrenals per kilogram of original body-weight in mgrm.	Weight of adrenals per kilogram of final body-weight in mgrm.	Total adrenalin in glands in mgrm.	Total adrenalin per kilogram of original body-weight in mgrm.	Total adrenalin per kilogram of final body-weight in mgrm.
227	250	170	60	240	352	0·102	0·408	0·600
244	210	160	47	223	293	0·090	0·428	0·562
232	260	180	58	223	322	0·150	0·576	0·833
254	230	140	76	330	542	0·129	0·560	0·902
253	180	130	57	316	438	0·120	0·666	0·923
229*	230	190	110	478	578	0·105	0·456	0·552
247	230	135	72	313	533	0·105	0·456	0·777
233	270	170	90	333	529	0·120	0·444	0·705
236	240	170	35	145	205	0·120	0·500	0·705
245	220	140	71	322	507	0·165	0·750	1·178
Average ...	232	158	67·6	292	429·9	0·1206	0·5244	0·7737

* Streptococcic infection.

Analysis of Table II:—

(1) In 12 cases of *dry* beriberi in this series the weight of the adrenals per kilogram of original body-weight ranged between 85 and 275 mgrm.; the weights of the adrenals were within the limits of health in two cases only.

When calculated against the final body-weight of the birds, the weight of the adrenals per kilogram ranged between 123 and 423 mgrm.; in no case was the weight of the adrenals within the limits of health.

(2) The total amount of adrenalin in the adrenal glands from these cases ranged between 0·045 and 0·138 mgrm. In 10 cases out of 12 the total amount of adrenalin was within the limits of health, but on a higher average than that of health.

(3) The average adrenalin-content per gramme of gland in 12 cases of *dry* beriberi was 0·0021 grm., or practically the same as in health.

(4) The total amount of adrenalin per kilogram of original body-weight ranged between 0·173 and 0·552 mgrm.; it was within the limits of health in 5 cases out of 12.

When calculated against the final body-weight of the birds, the total adrenalin ranged between 0·250 and 0·807 mgrm.; in all cases with one exception (No. 224), the total adrenalin per kilogram of body-weight exceeded the limits of health.

Analysis of Table III:—

(1) In 10 cases of *wet* beriberi in this series the weight of the adrenals per kilogram of original body-weight ranged between 145 and 478 mgrm.; in no case was this weight within the limits of health.

When calculated against the final body-weight of the birds, the weight of the adrenals per kilogram ranged between 205 and 578 mgrm.; in no case was this weight within the limits of health.

(2) The total amount of adrenalin in the enlarged adrenals from 10 cases of wet beriberi ranged between 0·090 and 0·165 mgrm. In no case was the adrenalin-content within the limits of health, although in one case (No. 244) it was as low as the maximum limit found amongst 10 healthy birds.

(3) The average adrenalin-content per gramme of gland in 10 cases of *wet* beriberi was 0·0018, or slightly below that of health.

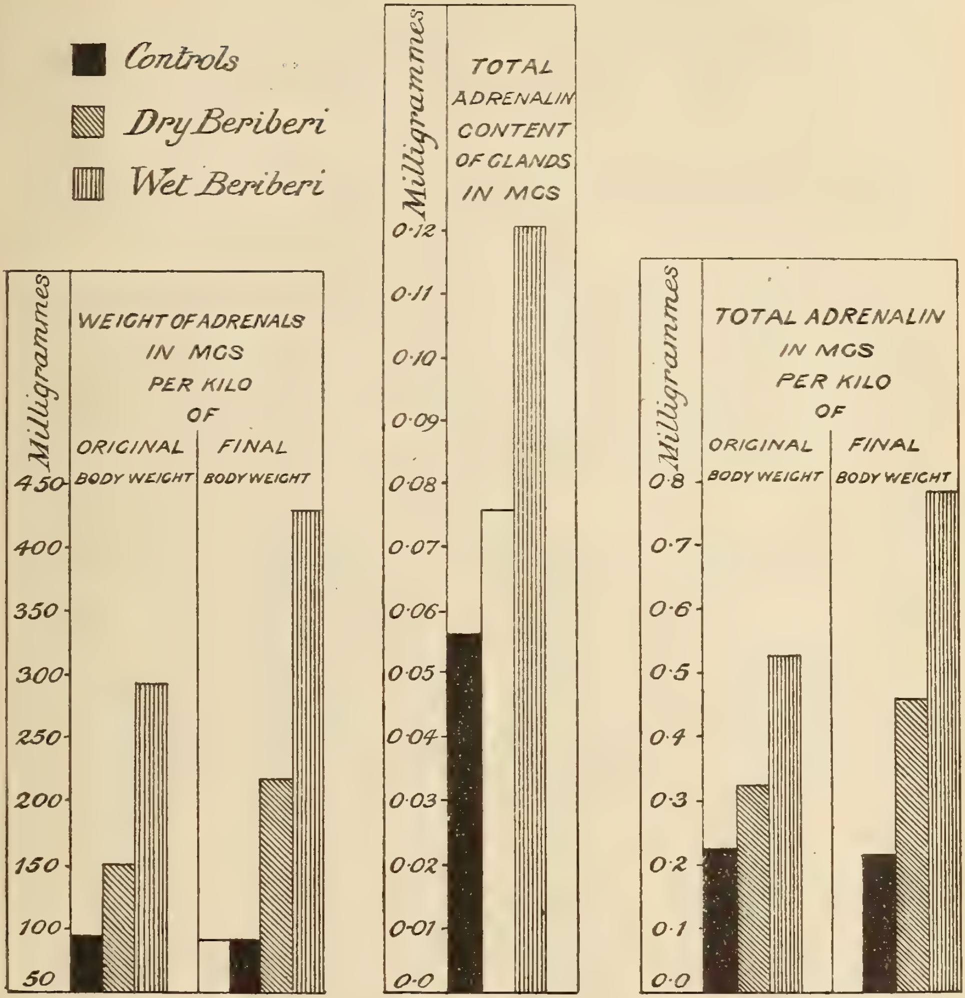
(4) The total amount of adrenalin per kilogram of original body-weight ranged between 0·408 and 0·750 mgrm.; in all cases, including No. 244 referred to in para. (2) above, it greatly exceeded the limits of health.

When calculated against the final body-weight of the birds, the total adrenalin in the body ranged between 0·552 and 1·178 mgrm., the excess over that found in health being very pronounced.

These findings are expressed as averages in the following Table, and are represented graphically in Chart I :—

Table IV.—Showing the Average Adrenalin-Content of the Adrenal Glands and the Average Quantity of Adrenalin per kilogram of Body-Weight in Healthy Pigeons and in Pigeons Suffering from Dry Beriberi and Wet Beriberi.

Original weight of pigeons in grm.	Final weight of pigeons in grm.	Weight of adrenals in mgrm.	Weight of adrenals per kilogram of original body-weight in mgrm.	Weight of adrenals per kilogram of final body-weight in mgrm.	Total adrenalin in glands in mgrm.	Total adrenalin per gram of gland in grm.	Total adrenalin per kilogram of original body-weight in mgrm.	Total adrenalin per kilogram of final body-weight in mgrm.	Class of pigeons.
245	255	23·2	94	91·8	0·0554	0·0023	0·226	0·216	Healthy.
239	169	36·0	151	216	0·0762	0·0021	0·321	0·456	Dry beriberi.
232	158	67·6	292	429	0·1206	0·0018	0·524	0·773	Wet beriberi.



Summary of Results.

(1) In the present series of cases of avian beriberi (polyneuritis gallinarum), œdema was found to be associated with massive enlargement of the adrenal glands. Case No. 236 (Table III) appeared to be an exception in so far as gross weight of these glands was concerned. It was found, however, that the adrenalin-content of the adrenals and the total adrenal per kilogram of body-weight were equal to or exceeded those of other cases of *wet* beriberi in which the gross weight of the adrenals was much greater.

(2) Two cases of *dry* beriberi (Nos. 243 and 246, Table II) had adrenal glands, and an amount of adrenalin per kilogram of body-weight equal to those of wet beriberi. Thus, 10 cases out of 12, or 83·3 per cent., having

massive enlargements of the adrenals or 0·408 mgrm. of adrenalin per kilogram of original body-weight or over (Tables II and III) had œdema in some form. This percentage is practically identical with that previously reported (1).

(3) The enlargement of the adrenal glands is a true hypertrophy in so far as the adrenal medulla is concerned: no conclusions are drawn with regard to the adrenal cortex.

(4) The adrenalin-content of the hypertrophied adrenals, as estimated by chemical methods, is slightly less per gramme of gland in cases of wet beriberi than in health or in dry beriberi.

(5) Nevertheless, in 100 per cent. of cases of wet beriberi the quantity of adrenalin greatly exceeds that found in health; in 83 per cent of cases it is also in excess of that found in dry beriberi.

Conclusions.

1. The conclusions previously reached (1) by physiological methods of adrenalin estimation are confirmed by chemical methods.

2. Deficiency of certain accessory food factors gives rise to a greatly increased production of adrenalin.

3. Whatever the function of the adrenal medulla may be, the excessive production of adrenalin under conditions of "vitaminic" deficiency is concerned with the causation of the œdema found in this order of cases. It must therefore be taken into consideration as a possible factor in the causation of œdema in general.

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Effect of Exercise and Humid Heat upon Pulse Rate, Blood Pressure, Body Temperature, and Blood Concentration.

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The effect of exercise on the human body has been made the subject of much study at different times. Researches have been carried out under atmospheric conditions such as prevail in different parts of Northern Europe, and they have been extended in a few instances to the effects of high temperature and humidity upon the human body. In the latter observations the conditions such as high temperature and varying humidity were produced by artificial means only, and general deductions as to the influence of an actual tropical climate upon the human organism cannot be safely drawn from them. In these experiments the subjects were living in a temperate climate, were exposed to heat and humidity for a short time only, and left the hot chamber at the end of the experiment for an atmosphere of coolness and comfort; in the tropics, on the other hand, the inhabitants are continuously exposed to heat and humidity without respite. Conclusions of real value can thus be drawn only from observations actually carried out in a hot climate, and systematic work in this direction is still lacking.

Although observations have been made in the tropics on body temperature, blood pressure, pulse and respiration rate, and metabolism, yet their object has only been to obtain normal standards for the tropics for comparison with those of Europe.

The following experiments were carried out in Townsville during the hottest months of the year (January to March), during which time the wet-bulb temperature stands between 75° and 80° F., occasionally even above, and the dry-bulb temperature between 80° and 90° F.; the degree of saturation of the atmosphere is very high, and the climate "trying." The climatic conditions—rainfall and temperature—in Townsville correspond, according to Griffith Taylor (1918), to those in Calcutta, with the exception that the humidity is slightly lower.

Methods.

The observations recorded were made on the staff of the Institute (subjects I to VI and VIII), and extended over two wet seasons. During the early part of the second year one of the authors (W. A. O., subject VII), during a visit to Townsville, joined in the research. The experiments were of three kinds. In a first series the effect of vigorous exercise of short duration was studied. The effort consisted in running up and down a staircase, about 15 feet high, as fast as possible; with few exceptions the feat was repeated 10 times, and the time taken varied from 118 to 150 seconds.

The second series comprised a number of experiments, in which the exercise consisted in walking for a varying period at a moderate pace (three to four miles an hour) during the hottest hours of the day. In one walking experiment on a very hot day (dry bulb 86·4°, wet bulb 81·0°), the observations were taken on three subjects (I, II, and III) at quarter-hourly intervals for two hours (the duration of the walk), and this experiment is treated separately, the observations being plotted in the accompanying chart.

In a third series of observations the effect of extremely high and humid

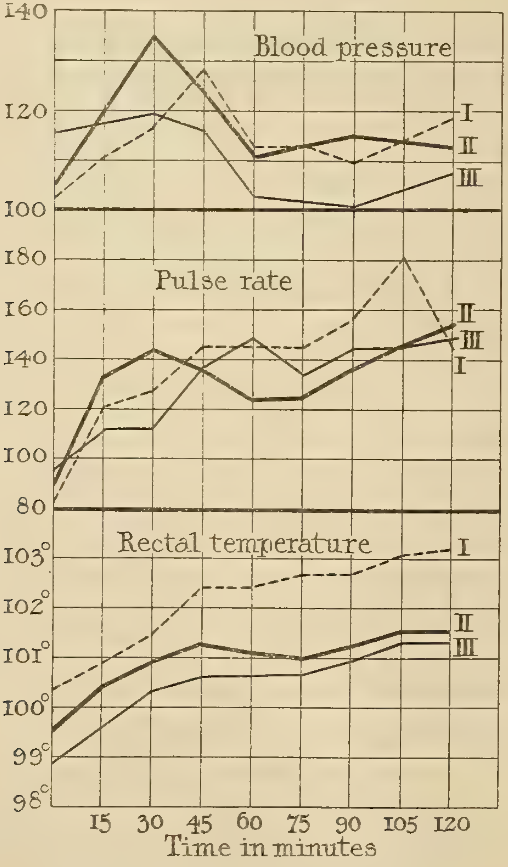

Chart of Walking Experiment. (See text.

heat was investigated upon three subjects, with a view to comparing the physiological effects produced by external heat alone with that produced by exercise in a hot climate. A small chamber of galvanised iron was used as a hot room. The iron was exposed to the direct sun's rays, and additional moist heat was produced by boiling water within the chamber: on sunny days a wet bulb temperature of about 102° F. could be obtained. The results are tabulated separately in Table VII.

In all experiments observations were made upon the changes of pulse rate, blood pressure, body temperature, and carbon dioxide content of the alveolar air. The pulse was counted for several quarter-minute periods, and in general the pulse rate of the subject in a lying position was contrasted with that of the subject standing up.

The blood pressure was estimated by means of a Tycos sphygmo-manometer, which instrument had been checked against a mercury manometer. The body temperatures recorded denote invariably rectal temperatures. The alveolar air was sampled by the Haldane-Priestley method.

Pulse Rate.—The pulse rate (Table I) in the subject at rest was within the range considered normal for a temperate climate, varying between 68 and 98 per minute. The pulse rate determined in an upright and recumbent position showed now and again a considerable difference, being lower in several of the subjects when lying down; considerable individual variations in this respect were noted.

The increase caused by exercise corresponded roughly with the violence of the effort, and in those experiments in Table I where the subject raced up and down stairs, the rise accorded with the length of time taken in performing the task. The pulse rate began to fall again immediately the exercise was discontinued, rapidly at first and more slowly afterwards, and after five minutes or so the pulse rate was still above the rate at rest.

In the walking exercises, extending over a prolonged period, the pulse rate at the end of the experiment was, as a rule, high, and it took a much longer time to return to the original rate. In a few instances the pulse rate was determined after half the walk had been accomplished, and it was observed that during the second half of the exertion practically no further rise was produced. In the special walking experiment (see chart) the pulse of the subjects I and II showed a rapid and marked rise at first; afterwards the pulse rate continued to rise at a much slower rate, or exhibited only insignificant variations.

In the hot room experiments the pulse rate rose very slowly and gradually concomitantly with the body temperature.

Blood Pressure.—The results of the estimations of blood pressure are

Table I.

No.	Subject.	Nature of exercise.	Pulse rate at rest.		Pulse rate after exercise.			
			Standing.	Lying.	Calculated from first 15 secs.	Counted during first min.	Counted in successive periods of 15 secs.	Rate after mins. counted in 15 sec. period.
		Running up and down stairs—						
1	I.	10 times in 155 secs.	80	—	128	124	32, 32, 28, 30, 30, 28, 27, 27	3 mins. 26; 18 mins. 23.
2		10 ,, 121 ,,	76	68	148	137	37, 34, 34, 32, 31, 31, 30, 28	5 ,, 25; 9 mins. 23.
3		10 ,, 129 ,,	72	72	136	126	34, 33, 30, 29, 29, 27, 27	5 ,, 25.
4		10 ,, 125 ,,	80	75	148	138	37, 35, 34, 32, 29, 28, 28	10 ,, 24.
5		10 ,, 127 ,,	74	72	148	133	37, 35, 32, 29, 29, 27, 27, 25	6 ,, 25.
6		10 ,, 123 ,,	76	72	160	143	40, 37, 34, 32, 29, 27	5 ,, 24.
7		10 ,, 118 ,,	70	68	176	152	44, 40, 36, 32	5 ,, 24.
8	II.	10 ,, 152 ,,	96	75	164	149	41, 37, 36, 35, 32	5 ,, 28; 8 mins. 25.
9		10 ,, 133 ,,	84	80	172	157	43, 40, 37, 37, 33, 32, 32, 30	5 ,, 26.
10		10 ,, 138 ,,	94	80	168	143	42, 37, 34, 30, 32, 30, 29	7 ,, 26.
11		10 ,, 130 ,,	98	80	164	151	41, 38, 37, 35, 32, 30	5 ,, 24.
12		10 ,, 123 ,,	84	69	144	141	36, 35, 35, 35, 32, 29, 30, 28	5 ,, 24.
13		10 ,, 118 ,,	90	78	164	148	41, 38, 35, 34, 31, 30, 30	5 ,, 23.
14	III.	7 ,, 90 ,,	80	76	164	153	41, 39, 37, 36, 37, 33, 33	4 ,, 29; 13 mins. 28.
15		8 ,, 125 ,,	78	72	172	158	43, 42, 37, 36, 35	5 ,, 26.
16		8 ,, 115 ,,	80	76	160	148	40, 36, 36, 36, 35, 34	5 ,, 30.
17		8 ,, 118 ,,	82	74	172	160	43, 41, 40, 36, 35, 35, 32, 30	5 ,, 26.
18		8 ,, 120 ,,	82	72	160	148	40, 36, 37, 35, 33, 32	5 ,, 25.
19		9 ,, 141 ,,	78	66	164	154	41, 40, 38, 35, 33, 32	5 ,, 27.
20	IV.	10 ,, 130 ,,	80	80	160	145	40, 37, 34, 34, 34	5 ,, 30.
21		10 ,, 150 ,,	72	72	140	125	35, 32, 30, 28, 26, 26, 24	6 ,, 22.
22		10 ,, 157 ,,	70	70	140	128	35, 32, 31, 30, 29, 27	5 ,, 20.
23		10 ,, 155 ,,	74	64	148	132	37, 34, 32, 29, 28, 24	5 ,, 19.
24		10 ,, 149 ,,	96	82	120	113	30, 29, 27, 27, 26, 24	5 ,, 24.
25		10 ,, 142 ,,	84	70	152	127	38, 34, 29, 26, 22	5 ,, 21.
26	V.	10 ,, 135 ,,	88	72	156	138	39, 36, 32, 31, 30, 30, 29, 28	5 ,, 25.
27		10 ,, 148 ,,	80	66	144	123	36, 31, 29, 27, 27, 26	5 ,, 26.
28		10 ,, 135 ,,	68	60	152	131	38, 34, 30, 29, 27, 26, 25, 24	5 ,, 24.

29	VI.	10 times in 130 secs.	80	68	164	147	41, 37, 35, 34, 34	5 mins. 25.
30		10 ,, 117 ,,	80	76	152	125	38, 38, 27, 22, 23, 23	5 ,, 25.
31		10 ,, 117 ,,	78	78	148	123	37, 35, 27, 24, 23	5 ,, 25.
32		10 ,, 110 ,,	94	82	180	129	45, 29, 28, 27, 27	5 ,, 25.
33	I.	Walking for 36 mins.	80	76	128	120	32, 31, 28, 29, 29	10 ,, 24.
34		Walking for 37 mins.	74	72	140	—	35, 34.	
		2nd walk of 33 mins.	—	—	160	133	40, 35, 30, 28	20 ,, 27.
35	II.	Walking for 30 mins.	92	80	160	—	40, 39.	
		2nd walk of 32 mins.	—	—	164	—	41, 38, 36	4 ,, 34.
36	IV.	Walking for 34 mins.	88	74	140	—	35, 32, 32.	
		2nd walk of 36 mins.	—	—	144	126	36, 32, 30, 28, 25, 27, 27.	
37	V.	Walking for 38 mins.	80	68	140	123	35, 32, 28, 28.	
		2nd walk of 38 mins.	—	—	132	119	33, 30, 28, 28, 26.	
38	II.	Climbing hill 1000 feet	84	—	148	—	37, 34, 33.	
39	III.	Climbing hill 1000 feet	84	—	180	—	45, 38, 37.	
		After descending same	—	—	160	—	40, 39.	

recorded in Table II. The figures indicate that the blood pressure was raised to a greater extent by violent exercise of short duration than by a prolonged and less strenuous effort. In the former series of experiments the blood pressure began to fall immediately after the cessation of exercise, and returned within a comparatively short time (about ten minutes) to the original. In the case of prolonged exercise the blood pressure rose at first considerably, and afterwards continued to rise very gradually or even remained stationary. In some instances the initial rise was followed by a fall to the normal, or sometimes even to a sub-normal figure. The curves in the chart illustrate this fact clearly. This fall in pressure is probably due to the dilatation of the cutaneous blood vessels brought about by the high temperature of the skin.

Table II.

No.	Subject.	Nature of exercise.	Blood-pressure (mm.).		
			Before.	Immediately after exercise.	After *n* minutes.
		Running up and down stairs—			
1	I	10 times in 129 secs.	109	152	5 mins. 139 ; 13 mins. 115.
2		10 ,, 123 ,,	105	162	5 ,, 126.
3		10 ,, 118 ,,	108	140	5 ,, 125.
4	II	10 ,, 152 ,,	112	172	8 ,, 122.
5		10 ,, 132 ,,	106	140	5 ,, 114.
6		10 ,, 118 ,,	110	145	5 ,, 123.
7	III	7 ,, 90 ,,	119	143	5 ,, 122.
8		8 ,, 125 ,,	112	162	3 ,, 146 ; 5 mins. 130. 8 mins. 108.
9		8 ,, 118 ,,	110	148	5 ,, 126.
10	IV	10 ,, 130 ,,	137	186	5 ,, 172 ; 8 mins. 140.
11		10 ,, 150 ,,	147	178	2 ,, 157 ; 5 mins. 142.
12		10 ,, 155 ,,	129	192	5 ,, 128.
13		10 ,, 142 ,,	132	162	5 ,, 130.
14	V	10 ,, 135 ,,	124	148	5 ,, 130.
15		10 ,, 130 ,,	122	170	5 ,, 135 ; 10 mins. 128.
16	VI	10 ,, 117 ,,	126	185	5 ,, 152.
17		10 ,, 110 ,,	141	176	5 ,, 132.
18	I	Walking 36 mins.	109	129	15 ,, 110 ; 45 mins. 109.
19		,, 37 ,,	119	130	
		Second walk in 33 mins.	—	148	20 ,, 118.
20	II	Walking 30 mins.	113	137	
		Second walk in 32 mins.	—	130	5 ,, 112.
21	IV	Walking 34 mins.	139	153	
		Second walk in 36 mins.	—	130	
22	V	Walking 38 mins.	112	132	
		Second walk in 38 mins.	—	128	
23	I	Climbing hill (1000 feet)	109	134	
		Descending same	—	118	
24	II	Ditto	110	135	
			—	123	
25	III	Ditto	112	102	
			—	102	

The observations upon the effect of humid heat alone upon the blood pressure were only carried out on two subjects, and gave inconsistent results. In some instances the blood pressure of one of the subjects rose, whereas it fell to a slight extent with the other. Hill and Flack (1909) on the other hand, observed a constant and considerable fall in the blood pressure after a stay of fifteen minutes in a hot bath of 100° to 112° F.

Body Temperature.—Previous observers have proved that the rectal temperature may rise to as much as 102° F. in perfectly healthy individuals as the result of moderate exercise in a temperate climate, and Hill and Flack have recorded even much higher temperatures in athletes. In one individual, for example, a temperature of 105° was recorded after a foot-race over a course of three miles, and these authors drew attention to the fact that the temperature rose with the duration of the effort.

In the present experiments exertion of short duration did not produce any rise of any consequence; 0·3°–0·8° F. was the usual elevation, and only on one occasion (subject VI) a rise of 2° was found.

Table III.

No.	Subject.	Nature of exercise.	Air temperature.		Rectal temperature.	
			Dry bulb.	Wet bulb.	Before exercise.	After exercise.
1	I	Walking 36 mins.	86·6	72·2	99·6	100·9
2		„ 37 „	87·3	76·0	99·4	101·3
		2nd time in 33 mins.	—	—	—	102·0
3	II	Walking 30 mins.	88·1	79·8	99·7	102·7
		2nd time in 32 mins.	—	—	—	103·0
4	IV	Walking 34 mins.	85·0	76·2	99·8	101·8
		2nd time in 36 mins.	—	—	—	102·0
5	V	Walking 38 mins.	84·2	74·3	99·9	101·3
		2nd time in 38 mins.	—	—	—	101·6
6	I	Walking 2½ hours	80·1	78·2	100·3	101·9
7		„ 3½ „	85·2	75·3	99·8	101·4
8		„ 2 „	86·4	81·0	100·3	103·1
9	II	„ 2½ „	80·1	78·2	99·8	101·2
10		„ 3½ „	85·2	75·3	99·5	100·6
11		„ 2 „	86·4	81·0	99·5	101·4
12	III	„ 2 „	86·4	81·0	98·8	101·3
13	VII	„ 2½ „	80·1	78·2	100·0	100·9
14		„ 3¼ „	85·2	75·3	99·6	101·0
15	I	Climbing hill (1000 feet)	86·2	78·1	99·6	102·0
16	II	„ „ „	86·2	78·1	99·7	102·0
17		„ „ „	85·0	75·5	99·5	101·4
18	III	„ „ „	86·2	78·1	99·2	103·3

Prolonged and less vigorous exertion—such as walking or hill-climbing during the hot hours of the day—caused much higher rectal temperatures, and the increase was more marked during the first part of the exercise than later. For example, in the experiments where the rectal temperatures were taken half-way through the walk, the rise during the first part amounted to 2° or 3° F., whereas it was only further increased by two or three-tenths of a degree after the repetition of the effort. The chart contains a graphic representation of the rectal temperatures during a two hours' walk on a hot day, and illustrates the above statement.

In the hot room a continued rise in rectal temperature was noted. This took place in the beginning slowly and gradually, but the rate increased with time and with increased temperature, thus confirming Haldane (1905), and Harvey Sutton's (1908) observations.

Alveolar air.—The percentage of carbon dioxide in the alveolar air has been estimated by a number of observers by the Haldane and Priestley method, and their results on individuals at rest have been found fairly constant. In Table IV are given the averages of 58 observations on seven subjects at rest, whereas Table V gives for comparison averages, maxima and minima, obtained by other workers in Europe. The figures obtained in Townsville are lower than those of the other authors, and this may be attributed to the influence of the higher temperatures of the atmosphere.

Table IV.—Alveolar Air during Rest.

Subject.	No. of observations.	Carbon dioxide percentage.		
		Average.	Maximum.	Minimum.
I.	8	4·58	4·96	4·03
II.	34	5·04	5·45	4·71
III.	4	4·76	5·0	4·60
IV.	3	5·33	5·86	5·01
V.	3	5·08	5·53	4·48
VI.	1	5·25	—	—
VIII.	5	4·77	4·95	4·50
Mean	58	4·99	—	—

Table V.

Observer.	No. of subjects.	Carbon dioxide in alveolar air.		
		Mean.	Maximum.	Minimum.
Haldane and Fitzgerald (1905)	27	5·59	6·34	4·72
Hill and Flack (1909)	17	5·32	6·35	4·05
Cook and Pembrey (1913)	10	5·57	6·11	4·87
Present observations	7	4·99	5·86	4·03

In this connection it has been noticed by Boycott and Haldane (1908) that outer temperatures influence the carbon dioxide in the alveolar air, and that there existed in fact a seasonal variation, higher percentages being found in cold and lower in warm weather. They do not believe, however, that these changes are caused by variations in the body temperature, but are solely due to the effect of contact of the face and hands with cold or warm air. Lower averages than the European figures were obtained by Chapman and Wardlaw (1916) working in Sydney, and Chapman has stated that he has never obtained a maximum higher than 6 per cent., which in his opinion is due to the higher average outer temperatures in Sydney.

Table VI contains the details of analyses of alveolar air after various forms of exercise. The percentage of carbon dioxide in general was greatly increased. After prolonged exercise the increase was much less marked than after vigorous exercise, and in experiments where an analysis was made halfway through the experiment the increase was more marked in the first than in the second half. In several experiments the analysis after exercise showed an actual decrease when compared with the figure at rest, which decrease may be attributed, as previously explained by Cook and Pembrey

Table VI.

No.	Subject.	Nature of exercise.	CO_2 per cent. in alveolar air.	
			Before.	After.
1	I.	Short vigorous 121 secs.	4·56	7·16
2		" 125 "	4·39	7·28
3		" 127 "	4·86	6·93
4	II.	" 133 "	4·82	6·03
5		" 138 "	5·02	5·63
6		" 133 "	4·83	5·43
7	III.	" 105 "	4·62	5·33
8		" 120 "	4·60	5·40
9		" 141 "	4·82	6·15
10	IV.	" 151 "	5·12	7·12
11		" 149 "	5·01	6·96
12	V.	" 148 "	4·48	8·32
13		" 135 "	5·53	8·16
14	VI.	" 117 "	5·25	9·05
15	I.	Walking 36 mins.	4·66	5·38
16		" 37 "	4·82	5·27
		2nd time in 33 mins.	—	5·44
17		Up and down hill 48 mins.	4·40	4·15
18	II.	Walking 30 mins.	5·03	4·26
		2nd time in 32 mins.	—	4·65
19		Up and down hill 48 mins.	5·01	4·08
20	III.	" " "	5·0	3·50
21	IV.	Walking 34 mins.	5·86	6·58
		2nd time in 36 mins.	—	5·95
22	V.	Walking 37 mins.	5·23	6·52
		2nd time in 38 mins.	—	6·62

(1913) to the washing out of carbon dioxide from the lungs by the vigorous ventilation.

In the hot room experiments the percentage of carbon dioxide did not show any constant variation, whereas Hill and Flack (1909) in their hot-bath experiment, observed a marked decrease in the carbon dioxide tension, owing to the increased rate of respiration due to the heat.

Loss in body weight.—The temporary loss in body weight brought about by exercise, and which is mainly due to loss of water, is naturally considerable in the tropics, on account of the increased perspiration. As shown in Table VII the loss bears relationship to the effort, time, and external heat. It was quite a common occurrence to lose as much as 1 kgrm. in weight during a walk of one hour at a moderate pace, and in one instance as much as 3200 grm. (Experiment No. IV in Table) was lost during a two hours' walk. The loss of weight in the hot room amounted to between 500 and 1200 grm. in one hour. In several exercise experiments quoted in the Table, the subjects were weighed in their clothing so that the loss represents only the water evaporated and not the total water lost from the body.

The loss of weight represents in the main loss of water secreted as perspiration; a certain amount of water is lost in the expired air, but as the air inspired is not far from the saturation point this amount is small. The loss in weight due to respiratory exchange may be neglected.* In the hot room, where the humidity was very high, practically the total loss in weight is due to perspiration.

The question as to the source from which the perspiration is derived has been discussed by Hunt (1912), who estimated the percentage of hæmoglobin in the blood before and after prolonged sweating in a hot room. He found that the hæmoglobin figures remained constant, and concluded from this that no concentration of the blood had taken place and that therefore the water excreted in the sweat was derived from the tissues and not from the blood serum. A similar experiment was carried out by Haldane and Priestley (1916)

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* In the course of another investigation by one of us (W. J. Y.) the respiratory exchange of two of the subjects was determined by means of a Zuntz portable meter, during a walk of 15 minutes at a rate of 3 miles per hour, and the figures obtained may be quoted here to show that only an insignificant portion of the weight lost is accounted for in this way.

In this experiment carbon dioxide was evolved at the rate of 107 and 119 grm. per hour by the two subjects respectively, and oxygen absorbed at the rate of 96 and 108 grm. per hour. The loss in weight due to respiratory exchange was therefore only 11 grm. per hour in each case.

In another experiment, one subject during an hour in the hot chamber lost about 7 grm. in weight due to the respiratory exchange.

Table VII.

No.	Subject.	Temperature of room.		Rectal temperature.		Pulse rate.				Blood-pressure.		Alveolar CO_2.		Duration of experiment.
		Dry bulb.	Wet bulb.	Before.	After.	Before lying.	Before standing.	After lying.	After standing.	Before.	After.	Before.	After.	mins.
1	I	100 ·1 to 101 ·2	95 ·8 to 98 ·0	100 ·1	102 ·2	—	76	—	124	120	123	4 ·96	4 ·04	60*
2		100 ·1 ,, 100 ·8	96 ·1 ,, 97 ·3	100 ·3	102 ·7	84	88	89	128	109	100	4 ·03	4 ·05/4 ·23	70†
3		100 ·1 ,, 107 ·0	98 ·7 ,, 102 ·0	99 ·8	102 ·0	—	—	—	132	—	—	—	—	40‡
4		93 ·0 ,, 95 ·0	88 ·5 ,, 92 ·0	99 ·8	100 ·2	—	—	—	—	—	—	—	—	60
5		96 ·2 ,, 97 ·1	91 ·2 ,, 95 ·2	99 ·5	100 ·4	66	74	62	78	110	103	—	—	60
6	II	94 ·8 ,, 100 ·3	98 ·2 ,, 96 ·0	99 ·5	103 ·0	72	72	112	168	112	142	—	—	78§
7		102 ·1 ,, 104 ·1	93 ·0 ,, 96 ·2	99 ·5	100 ·8	—	88	—	116	—	—	—	—	45
8		100 ·3 ,, 99 ·0	93 ·0 ,, 96 ·7	99 ·2	101 ·4	—	86	—	132	—	—	—	—	60
9		101 ·4 ,, 99 ·8	96 ·1 ,, 98 ·8	99 ·8	102 ·8	—	98	—	130	103	118	—	—	60
10	VII	106 ·0 ,, 108 ·0	92 ·0 ,, 95 ·0	99 ·7	100 ·8	—	90	—	128	—	—	—	—	60

* Throbbing in head.
† S.G. of blood—before 1 ·0535, after 1 ·0567.
‡ Fainting.
§ S.G. of blood—before 1 ·0515, after 1 ·0552.

who failed to detect any alteration in the percentage of hæmoglobin in the blood after a net loss of 1·07 kgrm. in body weight.

Experiments were undertaken to ascertain whether the estimation of the specific gravity of the blood might yield further information on the source of the sweat. Observations on the specific gravity of the blood were carried out some time ago in this Institute by Hammerschlag's chloroform-benzene method and yielded figures well within the range of European estimations. The same method was employed to estimate the specific gravity of the blood before and after profuse sweating, in order to ascertain whether any concentration could be detected by this means. Although the estimations showed a very slight increase in the specific gravity of the blood after sweating in the hot room, the differences were within the range of experimental error, which is considerable. Unless a mixture of the same specific gravity as that of the blood were hit upon immediately it was difficult to obtain a constant behaviour of a drop of blood; moreover the size of the drop had an undoubted effect upon the result. For the above reasons this method was abandoned, and in the later experiments the total solids of the blood before and after the sweating and the refractive index of the blood serum were estimated.

For the total solids a few drops of blood oozing out freely from a deep puncture wound in the lobe of the ear were collected on a small piece of thick blotting paper, previously dried and tared. It was quickly weighed and dried to constancy at 110° C. The amount of blood collected weighed about 120 mgrm. to 130 mgrm., and the weighings were done by a specially constructed micrometer balance, designed and made by Messrs. Felton, Grimwade and Company of Melbourne, accurate to 1 mgrm., which enabled the paper to be weighed in a few seconds; the whole determination could thus be completed in 15 to 20 minutes. All estimations were done in duplicate.

The method was not found altogether reliable on account of the possible sources of error. If the blood did not flow freely from the puncture wound and pressure had to be resorted to, the estimations of the blood solids gave inconsistent results. In addition, there was always the possibility of a small amount of sweat being taken up simultaneously with the blood by the paper.

In spite of these possible errors, after a few preliminary experiments duplicate estimations gave fairly consistent results, and the calculated percentage of the total solids in the blood (about 20 per cent.) from two separate estimations did not differ by more than one.

The total solids in the blood showed a decided tendency towards increase after perspiring; out of 11 experiments, in six the increase was larger than the experimental error, varying between 1·5 and 3·1. In three experiments there was an increase, but within the error limit, and an actual but small decrease was observed in two experiments. The increase in the blood solids in the different experiments was not proportionate to the loss in body weight. In the detailed Table a column is given indicating the loss of water from the blood, which would correspond to the increase in the solids actually observed. Considering the small actual difference in the weighings, these calculations are only very approximate, and only the one conclusion is justified—that there is a tendency to increased blood solids brought about by copious sweating.

The refractive index of the serum was measured before and after copious perspiration. The blood was collected from the ear by means of a Wright's tube, allowed to clot and centrifugalised, and the serum examined by means of a Zeiss-Abbé refractometer. In a few instances serum of blood obtained from the ear and from the finger were compared and yielded identical results. In every case the refractive index of the serum was higher after profuse sweating, whether brought about by exercise or merely by exposure to excessive moist heat as shown in Table VII.

In the Table a column of figures is given which represents the percentage of water lost from the blood, calculated from the increase in the refractive index. The figures were arrived at in the following manner: A series of weighed quantities of goat's blood was allowed to evaporate slowly in a desiccator over sulphuric acid, and the increase in the refractive index of the serum was then plotted against the corresponding loss of water from the blood, and a curve smoothed through the points. From this curve the percentage of loss of water from the blood corresponding to the increase in the refractive index of the serum could be obtained.

On comparing the total loss in body weight with the calculated loss of water from the blood, based on the refractive index of the serum, it is clear that the greater part of the sweat must be derived from other sources than the blood, although the experiments show that at least part of the fluid lost is derived from the blood plasma.

It will be observed also from the Table that the quantity of water lost from the blood, as calculated from the refractive index, and that estimated from the increase in the total solids of the blood, do not agree, but, as pointed out previously, the errors in estimating the total solids were too great to consider this method more than a qualitative one.

The estimation of the refractive index gave such constant results that one must conclude that a definite concentration of the blood does take place as the result of copious sweating. The percentages, however, seem rather high,

Table VIII.

No.	Subject.	Nature of experiment.	Air temperature.		Body weight.	Loss.	Refractive index of serum.				Blood solids.			
			D.B.	W.B.			Before.	After.	Increase.	Water loss.	Before.	After.	Change.	Water loss.
					kilog.	grm.				p. c.	p. c.	p. c.	p. c.	p. c.
1	I.	Walking 36 mins.	86·6	72·2	69 clothed	750								
2		„ 37 „	87·3	76·0	68·8 „	610								
		2nd time in 33 mins. ...	—	—	—	550								
3		Climbing hill, 1000 feet	86·2	78·1	69 clothed	950								
4		Walking 2 hours	86·4	81·0	68 naked	3200								
5	II.	„ 30 mins.	88·1	79·8	48 clothed	300								
		2nd time in 32 mins. ...	—	—	—	440								
6		Climbing hill	86·2	78·1	47·6 clothed	560								
		Walking 2 hours	86·4	81·0	44·8 naked	1500								
8	III.	Climbing hill	86·2	78·1	79·3 clothed	1030								
9		Walking 2 hours	86·4	81·0	74·2 naked	2500								
10	IV.	Walking 34 mins.	85·0	76·2	61·25 clothed	400								
		2nd time 36 „	—	—	—	480								
11	V.	Walking 38 „	84·2	74·3	59·95 clothed	500								
		2nd time in 38 mins. ...	—	—	—	400								
12	I.	Hot chamber 60 mins. ...	100·6	96·9	67·25 naked	1540								
13		„ 60 „ ...	100·3	96·8	67 „	2000								
14	II.	„ 80 „ ...	97·7	92·1	45·4 „	650								
15		„ 60 „ ...	99·6	93·5	45 „	500								
16	I.	Walking 2½ hours	80·1	78·2	67 „	1530	1·3477	1·3481	0·0004	4·2	20·2	21·0	+0·8	3·8
17		„ 3½ „	85·2	75·3	67 „	2733	1·3469	1·3485	0·0016	8·8	19·2	22·2	+3·0	13·5
18	II.	Climbing hill	85·0	75·5	45 „	700	1·3470	1·3479	0·0009	6·9	22·2	20·7	−1·5	
19		Walking 2½ hours	80·1	78·2	45 „	693	1·3468	1·3478	0·0010	7·2	20·3	19·4	−0·9	
20		„ 3½ „	85·2	75·3	45 „	1502	1·3474	1·3482	0·0008	6·7	18·1	20·3	+2·2	10·8
21	VII.	Climbing hill	85·0	75·5	89 „	1265	1·3476	1·3480	0·0004	4·2				
22		Walking 2½ hours	80·1	78·2	89 „	1440	1·3467	1·3475	0·0008	6·7	20·4	21·9	+1·5	6·9
23		„ 3½ „	85·2	75·3	89 „	2730	1·3460	1·3480	0·0020	9·5	20·3	21·3	+1·0	4·7
24	I.	Hot chamber 35 mins. ...	107·0	100·3	67 „	1220	1·3473	1·3482	0·0009	6·9	19·0	21·4	+2·4	11·2
25		„ 60 „ ...	94·0	91·1	67 „	595	1·3475	1·3480	0·0005	5·0	19·2	20·2	+1·0	5·0
26	II.	„ 45 „ ...	102·9	94·6	45 „	506	1·3462	1·3470	0·0008	6·7	18·5	20·6	+2·1	10·2
27	VII.	„ 60 „ ...	101·0	93·5	89 „	970	1·3469	1·3481	0·0012	7·8	17·5	20·6	+3·1	14·8

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and in all probability the calculated percentages can only be regarded as rough approximations.

These results thus differ from those of Hunt, who was unable to detect any loss of water from the blood by means of hæmoglobin estimation, but bear out his contention that the bulk of water in the sweat is derived from the tissues. As an additional source of water, the intestinal canal must be kept in mind.

Office

Summary.

1. Vigorous exercise of short duration caused—

    (*a*) An increase in the pulse rate and blood pressure, both of which rapidly fell to normal after discontinuation of the exercise.

    (*b*) An increase in the carbon dioxide percentage of the alveolar air.

2. The alveolar air at rest in inhabitants of tropical Queensland showed a lower carbon dioxide content than the European average.

3. Prolonged exercise led to a rapid increase in the pulse rate and temperature at first, which increase became more gradual afterwards, and in the case of blood pressure even fell on occasions below normal, on account of the profuse sweating. Prolonged exercise had but little effect on the alveolar air.

The body temperature during the exercise continued to rise slowly, but, considering the light nature of the exercise, the rise in temperature was considerable.

4. A considerable loss of water from the body was observed as the result of prolonged exercise. Blood estimations showed that this water was mainly derived from other sources in the organism than the blood plasma; a small concentration of the blood plasma, however, had taken place.

5. The hot room experiments gave results similar to those caused by prolonged exercise, with this difference, that the pulse rate and body temperature rose more gradually at first, but a quicker rise took place afterwards.

6. The results point to the fact that both exercise and humid heat play a part in producing a rise in blood pressure, pulse rate, and rectal temperature. The degree of rise, however, is controlled by atmospheric conditions which influence the rate of cooling of the body.

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CROONIAN LECTURE.—*The Biological Significance of Anaphylaxis.*

By H. H. DALE, C.B.E., M.D., F.R.S.

(Lecture delivered May 29, 1919.—MS. received October 21, 1919.)

MR. PRESIDENT, LADIES, AND GENTLEMEN,—I am deeply sensible of the honour conferred upon me by the invitation to deliver the Croonian Lecture before this Society. In conveying that invitation the Council did me the further service of indicating a desire that the lecture should deal with "The Biological Significance of Anaphylaxis." From the wording of the title thus suggested, and from their choice to deal with this subject of one whose own activities have lain outside the conventional limits of immunological study, I gather that the Council's intention was that the lecture should deal with the interest of the phenomena of anaphylaxis for a wider field of biological enquiry than that to which their investigation primarily belongs.

You will not expect or desire that I shall attempt a detailed review of the enormous literature which has grown up, with almost unique luxuriance, round the study of anaphylaxis. I shall deal with the history of the investigation in summary fashion, mentioning few of the participants by name, and giving only such broad outlines as will serve to make clear the nature of the problem to any who may be imperfectly familiar with it. In presenting some of my own experiments in somewhat fuller detail, I am guided by what I believe to be the Council's desire, that I should put before you a personal and individual view, rather than embark on the hopeless endeavour to compress within the limits of a lecture the material for a judicial appreciation of the evidence.

Attention was first definitely drawn to the phenomenon now generally known as anaphylaxis by Richet* in 1902. He was investigating the action of a poisonous protein obtained from sea-anemones. Having on one occasion injected into the vein of a dog a dose small enough to cause only slight and evanescent symptoms, he gave another similar injection to the same animal some weeks later, expecting to find some degree of immunity to the poison. Contrary to expectation, this second injection caused an immediate and violent intoxication, to which the dog succumbed. The phenomenon was found to be of regular occurrence when this or other poisonous proteins were administered in this way, with a sufficiently long interval between the injections. It appeared to Richet to be the direct opposite of immunity or "phylaxis," and he named it "anaphylaxis." The name would be difficult to defend on the ground of etymology; it does not properly express even the imperfect conception which its author had in mind at the time; but usage and convenience have given it superabundant sanction.

The experiments of Arthus† on the increasing sensitiveness of rabbits to successive injections of horse serum, and the investigations following a further accidental discovery of the phenomenon by Theobald Smith,‡ in 1904, showed its nature in a truer light. It was noticed that guinea-pigs, which had some weeks previously received a very small injection of horse's blood serum reacted to a further injection, of this normally very innocuous substance, as to an acute poison. It soon became clear that anaphylaxis was not a state of diminished resistance to the action of a normally poisonous protein, but a condition in which a previously injected protein, whether naturally poisonous or not, acted like a poison of a very acute type, the symptoms produced being characteristic of the condition and of the species of animal exhibiting them, but in no way of the substance. If the latter had a natural toxicity the anaphylactic animal was, indeed, immune as regards this, reacting now to the substance simply as to any protein to which it had been rendered specifically sensitive.

General interest being now aroused in the phenomenon, it was recalled that Magendie had already recorded its occurrence in 1839, and that several other observers had given it incidental mention at later dates, without having realised its meaning and importance. Its relation to certain conditions seen in man began also to be recognised—asthma provoked by the exhalations of certain animals, hay fever, and analogous idiosyncrasies. In 1905 v. Pirquet and Schick* had described the accelerated and intensified reaction to horse serum in patients receiving a second therapeutic injection. Richet's further experiments, and others in America and Germany following Theobald Smith's discovery, quickly established the main characteristics of the condition. An interval of one to several weeks, varying with the substance and the animal, must elapse after the first injection before a second would produce the abnormal reaction; an additive or cumulative effect was therefore out of the question. The sensitiveness was highly specific; if an animal received a first injection of horse serum, for example, it became anaphylactic to that serum only, and not to that of a sheep or a man. If blood or serum from an animal which had been rendered sensitive was injected into a normal animal, the specific sensitiveness was transmitted to the latter, which was rendered "passively anaphylactic."

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* 'C. R. Soc. de Biol.,' 1902.

† 'Bull. Soc. Biol.,' 1903.

‡ Theobald Smith, 'Journ. Med. Res.,' 1904; Otto, 'v. Leutold Gedenkschr.,' 1906; Rosenau and Anderson, 'Hyg. Lab. Bull.,' Washington, No. 29, 1906; No. 36, 1907; No. 38, 1907.

In all these respects anaphylaxis closely resembles immunity. To use the terms of immunology, the substance in the preparatory injection acts as an "antigen," evoking the appearance of some new constituent or "antibody" in the blood or tissues of the subject; on the presence of this "antibody" depends the abnormal reaction to the second injection, and its transfer to a normal animal conveys the specific sensitiveness.

In the nature of the substances producing it, and in the limits of its specificity, anaphylaxis shows, moreover, a very striking resemblance to a particular type of immunity, associated with the production of a particular type of antibody. The typical anaphylactic antigens are the natural, large-moleculed proteins of cell-protoplasm, and of body-fluids such as blood or lymph. Attempts to demonstrate anaphylaxis to naturally occurring lipoid substances have provided no convincing evidence. The guinea-pig is the animal which has formed the subject of most of the experiments on this point. It occupies, as we shall see, a peculiar position in the ease and regularity with which it can be rendered anaphylactic, and in the dramatic intensity with which it reacts to a further injection of the antigen. The traces of protein which suffice to produce anaphylaxis in this species are so minute, that it is practically impossible to exclude them from lipoidal extracts of tissues. Carbohydrates have certainly no power of producing anaphylaxis.

I mentioned the native proteins as the typical anaphylactic antigens. Purification, however, does not diminish their activity in this direction.

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* v. Pirquet und Schick, 'Die Serumkrankheit,' Vienna, 1905.

Osborne and Wells* have demonstrated typical anaphylaxis to the crystalline proteins from various seeds, and to the crystallised albumin from white of egg.† Hartley and I‡ observed it with a very highly purified sample of crystallized albumin from horse-serum. Indeed, it would seem that purification of the protein diminishes the effective dose. Wells produced anaphylaxis in a guinea-pig with a preparatory injection of 1/20,000 mgrm. of crystallised egg-albumin, while 1/20 mgrm. of the same prepraration sufficed to kill a guinea-pig rendered anaphylactic to it.

While purification does not diminish the antigenic efficiency, any denaturating influence rapidly destroys it. Proteins which are coagulable by heat lose the action when their solutions are boiled; others, like casein, which are not coagulable, retain their antigenic properties after boiling. The earlier products of digestive hydrolysis probably retain the property to some extent, but it rapidly disappears as digestion proceeds, and is completely lacking in the free amino-acids. It is of special interest to note that racemization of a protein, by gentle warming with alkali, completely destroys its antigenic activity (Ten Broek§).

We have seen that the sensitiveness is highly specific. Injection of blood serum, for example, produces anaphylaxis to serum from the same species, but no abnormal sensitiveness to that from others; at most, a trace of sensitiveness to serum from closely related species can be detected. The reaction discriminates, however, not only between substances from different species, but between the constituents of different organs from the same species, and finally between different pure proteins from the same organ or tissue, as well as between corresponding pure proteins from different species. Wells‖ found that each of the five proteins from the hen's egg could produce a separate and more or less specific anaphylaxis in the guinea-pig. Hartley and I (*loc. cit.*) found that anaphylaxis discriminated between the three proteins separable from horse serum. A guinea-pig which received a preparatory injection of the pure euglobulin became sensitive to this, but remained indifferent to the pure albumin, and *vice versâ*. If the animal received a small preparatory injection of the whole serum, the sensitiveness to euglobulin appeared first (8–10 days), that to albumin only after an interval about twice as long (16–20 days). Even between the two globulins, the so-called eu- and pseudo-globulins, the discrimination was clear, though less rigid, possibly on account of the difficulty of purification.

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* 'Journ. Infect. Dis.,' vol. 8, p. 66 (1911); vol. 12, p. 341 (1913).

† 'Journ. Infect. Dis.,' vol. 5, p. 449 (1908).

‡ 'Biochem. Journ.,' vol. 10, p. 408 (1916).

§ 'Journ. Biol. Chem.,' vol. 17, p. 369 (1914).

‖ 'Journ. Infect. Dis.,' vol. 9, p. 147 (1911).

Now, in the nature of the substances which produce it, in the conditions which destroy, weaken or modify their action as antigens, and in the limits of its specific discrimination, anaphylaxis shows a remarkable and suggestive similarity to the well-known type of immunity dependent on the so-called "precipitin" reaction.[*] If any of the proteins which typically produce anaphylaxis is injected in suitably large doses into an animal, at intervals too short to allow the appearance of a definite anaphylaxis, the injections evoke a change in the blood serum and body fluids of such a nature that, when the serum or other fluid is mixed with a solution of the immunising antigen, often in very high dilutions, a mutual precipitation of the proteins occurs. The precipitin reaction discriminates between individual proteins, such as those from a serum, just as the anaphylactic reaction does. One other example, out of a vast number available, must suffice to illustrate the close correspondence in specificity between the two types of reaction. Among body proteins, that of the crystalline lens occupies a peculiar position as an antigen. Through a large range of species this protein acts as a common precipitating antigen. A precipitating serum, prepared by immunising an animal with lens proteins from the eyes of horses, will form precipitates equally well with lens protein from ox, cat, man, or other species, including even that to which the animal furnishing the serum belongs. The lens, cut off completely from the circulation, seems to behave in this respect as though its constituents were alien to the species. As an anaphylactic antigen the behaviour of the lens protein is exactly similar;[†] it is even on record that a guinea-pig has been rendered anaphylactic by an injection of protein from one of its own lenses, and subsequently poisoned by injecting protein from the other.

A large volume of indirect evidence of this kind strongly suggests that the antibody causing anaphylaxis is identical with the so-called precipitin. But there is one obvious and apparently fundamental difficulty in the way of assuming their identity. The serum of the anaphylactic guinea-pig contains no demonstrable precipitin; that is to say, it forms no visible precipitate when mixed with the sensitizing antigen. On the other hand the animal in which, as the result of repeated injections, a strong precipitating quality has been acquired by the serum, is not anaphylactic but immune.

The difficulty, however, is not so fatal as it appears. We have seen that anaphylaxis can be transmitted to a normal animal by serum from one which is itself anaphylactic. It is found, however, that serum from an immunized animal is even more effective in thus rendering a normal one passively anaphylactic, and that its efficiency, measured by the reciprocal of the volume required to communicate a given intensity of anaphylaxis, is closely parallel to the intensity of its precipitating quality.

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[*] *Cf.* Friedberger, 'Zeitschr. f. Immun.,' vol. 2, p. 208 (1909); Doerr and Russ, *ibid.*, vol. 3, p. 706 (1909).

[†] Uhlenhuth and Andrejew, 'Arb. aus d. k. Gesundheitsamt,' vol. 30, No. 2; Kraus, Doerr, and Sohma, 'Wien. klin. Wochenschr.,' vol. 21, p. 1084 (1908).

There is another feature of this production of passive anaphylaxis in the guinea-pig which is highly suggestive. When serum from an anaphylactic or immune animal is injected into a normal guinea-pig, even when the injection is made into a vein, no trace of the transmitted sensitiveness can be detected for a period of some hours, and it is not fully developed until a day or so after the injection. By a series of ingenious experiments the late Richard Weil,* of New York, was able to follow the fate of the antibody after such transference to the normal guinea-pig, and to show that the passive anaphylaxis did not begin to appear until the antibody began to leave the blood, and that most of the antibody had disappeared from the circulation by the time the sensitiveness was fully developed. When the animal had thus been rendered sensitive, a further large injection of the immune serum protected the animal for the time being against the otherwise fatal effect of an injection of the antigen.

All this evidence seems to point clearly in one direction. It strongly suggests that the so-called anaphylactic antibody and the precipitin of the immune serum are either identical or very closely similar; that the remarkable difference in physiological response to the antigen, between the anaphylactic and the immune animal, depends on the different distribution of the antibody between the blood and the cells of the vital organs; that the antigen acts as a poison to the anaphylactic animal because its reaction with the antibody takes place in the cells; but that an excess of antibody in the blood protects the cells, by removing the antigen from the sphere of action before it reaches them, and thereby renders the animal immune. This is the conception of anaphylaxis and its relation to immunity which I shall endeavour to commend to you with the aid of some experimental results of my own work. It was one of the earliest to be put forward, being known in the language of the side-chain theory as the theory of "sessile receptors." It must be admitted that the evidence in its support has been obtained almost entirely by experiments on the guinea-pig, and that there is reason for hesitation in applying it to species in which anaphylaxis has as yet been less thoroughly investigated. Experiments on the guinea-pig, however, supplied also the evidence advanced in support of other theories, which have largely displaced this conception in the estimation of investigators on the continent of Europe, and, to a less degree, in America. A very large proportion of the vast literature of the subject is devoted to their support and elaboration. Before dealing with these alternative conceptions, it is necessary to give some account of the physiological mechanism of the poisonous action of the antigen on the anaphylactic animal.

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* 'Journ. Med. Res.,' vol. 27, p. 525 (1913); vol. 29, p. 233 (1913).

The complex of symptoms, often spoken of as the anaphylactic shock, differs widely in the various species in which it has been observed, though the symptoms in each species are fairly constant. In the guinea-pig the picture is dominated by an asphyxiating obstruction of the respiration,* due to contraction of the plain muscle surrounding the bronchial tubes, which produces a valve-like closure of the lumen,† so that air can be sucked into the chest by violent effort, but cannot again be expelled. In the dog the main characteristic is a profound fall of blood-pressure, with congestion and hæmorrhagic œdema of the alimentary mucous membranes.‡ The liver in this species becomes enormously swollen and congested, and it has been shown, by Manwaring§ and others, that if the blood is diverted from the liver, the general effects on the circulation are weak or absent. In the rabbit the central feature appears to be a failure of the heart. There are, in addition, certain features common to all species, if the fatal termination is postponed long enough to permit their appearance—flushing and irritation of the skin, loss or impairment of the natural coagulability of the blood, scarcity of leucocytes and agglutination of the blood platelets, and rapid fall of body temperature owing to depression of the metabolism. The differences, however, are very striking, and would be puzzling, were it not that the contrasted symptoms represent, in each case, the characteristic reaction of the species to a large class of poisonous proteins and protein derivatives—peptones, protamines, bacterial proteins, and various extracts of animal organs. The main features in each case—asphyxiating bronchial spasm in the guinea-pig, collapse of the circulation in the dog—were found by Laidlaw and myself‖ to be produced also by the organic base, amino-ethyl-glyoxaline, produced from the diamino-acid histidine by decarboxylation, and usually known as "histamine." More recently, with the co-operation of Prof.

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* Auer and Lewis, 'Journ. Exp. Med.,' vol. 12, p. 151 (1910); Biedl and Kraus, 'Wien. klin. Wochenschr.,' No. 23, p. 385 (1910).

† Anderson and Schultz, 'Proc. Soc. Exp. Biol. and Med.,' vol. 7, p. 32 (1910); Schultz and Jordan, 'Journ. Pharmacol. and Exp. Therap.,' vol. 2, p. 375 (1911).

‡ Biedl and Kraus, 'Wien. klin. Wochenschr.,' No. 23, p. 363 (1909).

§ 'Zeitschr. f. Immun.,' vol. 8, p. 1 (1910).

‖ 'Journ. of Physiol.,' vol. 41, p. 318 (1910).

Richards* of Philadelphia, we analysed this type of action more fully, and were able to reduce the main features of the complex, as seen in different species, to an action predominantly on two tissues—stimulation of plain muscle fibres to intense tonus, and a poisoning of the endothelium forming the walls of the capillaries, with the result that the blood tended to collect at the periphery in the widened capillary spaces, while the plasma escaped through the abnormally permeable endothelial cells. With some poisons the injury is so severe that the blood itself escapes from the capillaries and passes into the tissues, as in cases of severe anaphylactic shock in the dog.

There are minor differences of distribution of the action of the different poisons of this class, but the fundamental community of the type is, I think, unmistakable. To this common type of toxic action, which obviously has a very wide pathological importance, the anaphylactic shock conforms. In the guinea-pig the action on the plain muscle of the bronchioles often produces such rapid death that the rest of the complex has not time to develop; in the dog the effect on plain muscle is relatively slight or wanting, and the main effect is that on the blood-capillaries, especially in the liver.

It is of special interest, for our immediate purpose, to note that substances having this type of action are liberated by injury of various cells and tissues, including the blood. The latter in clotting acquires an evanescent toxicity of this type, which is accentuated by rough treatment calculated to injure the corpuscles and platelets.

I may allow myself a momentary and very slight digression, to mention that during the war this type of action has come to light in a quarter not hitherto suspected, as at least an important factor in some of the conditions, loosely classified as "shock," following massive injury of the soft tissues, from which poisonous substances appear to be absorbed into the circulation.

This striking resemblance between the anaphylactic shock and the effects of certain protein derivatives, formed the basis of the alternative theories of the nature of anaphylactic shock, to which a very large proportion of the work on the subject has been devoted. These other theories have in common the supposition that a toxic substance or condition arises in the blood, to which the symptoms are immediately due. Some have supposed that the symptoms following the re-injection of the antigen were due to the liberation in the blood of toxic products of protein hydrolysis.† In an earlier form of this conception it was supposed that the formation of the complex of antigen and antibody brought the former into relation with a proteolytic ferment in the blood, and initiated its rapid digestion.* In a more recent form of the theory it is supposed that the formation of this complex removes the antitryptic factor present in normal blood, releases the action of the tryptic ferment, and thereby initiates an autolytic cleavage of the blood proteins.† Another type of theory regards the formation of the complex of antigen and antibody as disturbing the delicate equilibrium of the plasma colloids like contact with a foreign surface, thereby initiating changes analogous to those which precede clotting, and imparting to the blood a toxicity similar to that which appears during that process, or in some vaguer way rendering it toxic by disturbance of the state of colloidal solution.‡

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* 'Journ. of Physiol.,' vol. 52, pp. 110, 355 (1918).

† Richet, 'C. R. Soc. de Biol.,' vol. 61, p. 1005 (1909); Vaughan, 'Zeitschr. f. Immun.,' vol. 11, p. 673 (1911); also 'Protein Split Products,' New York, 1913.

If we consider the large mass of evidence put forward in support of these plausible and attractive hypotheses, we find that it almost all depends on the possibility of artificially enhancing the toxicity of blood serum *in vitro*. Serum freshly separated from clotted blood retains some of the toxicity which is developed during clotting, but much of this toxicity seems to have disappeared. This natural slight toxicity of a fresh serum can be enhanced, or revived, by treating it in various ways. Some of these have an apparent relation to the anaphylactic phenomena, as when serum is incubated with the specific precipitate formed by an antigen with a precipitating serum. In some the connection is remote, as when the serum is incubated with an emulsion of dead or living bacteria. Serum has been rendered toxic by shaking it with chloroform, by acidulating it and filtering through kieselguhr, by contact with kaolin, and with sols of silica, agar-agar, starch, inulin, or with heat-coagulated protein.§ In some cases the appearance of the toxicity is accompanied by autolytic cleavage of the serum proteins, in others, none can be detected. In some experiments with Walpole|| I found that autolysis of serum or plasma, brought about by removing the natural antitryptic factor, was accompanied by liberation of a thrombokinetic substance. Injection of such serum into a guinea-pig caused rapid death, with symptoms having a superficial resemblance to the anaphylactic shock; but the cause of death was a massive coagulation of blood in all the vessels. Serum rendered toxic by incubation with agar, on the other hand, which produces no perceptible autolysis, kills the

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* Friedberger, 'Zeitschr. f. Immun.,' vol. 4 (1909).

† Jobling and Petersen, 'Journ. Exp. Med.,' vol. 20, p. 37 (1914); Bronfenbrenner, 'Journ. Exp. Med.,' vol. 21, p. 480 (1915).

‡ Doerr, 'Kolle und Wassermann's Handbuch,' 1913, Art. "Anaphylaxie"; Novy and De Kruif, 'Journ. Infect. Dis.,' vol. 20, Nos. 5 and 6 (1917).

§ A review of experiments of this type is given by Sachs, 'Kolloid Zeitschr.,' 1919.

|| 'Biochem. Journ.,' vol. 10, p. 331 (1916).

guinea-pig in a manner much more closely resembling the anaphylactic shock. It may freely be admitted that a full understanding of the nature of the changes in serum, which give it the power of inducing these symptoms, might throw a great deal of light on our problem.

The weakness of all this evidence, however, seems to me to lie in the lack of clear connection with the conditions of the true anaphylactic reaction. Much of the work seems to have proceeded on the unconscious assumption that the symptom complex was in itself characteristic, and that the appearance of this type of toxicity was sufficient warrant for classing the phenomenon as anaphylactic. We have seen that the symptoms are not characteristic; the essential feature of anaphylaxis is that they are produced by a substance which normally has no such effect. If it could be proved that the injection of the antigen into the anaphylactic animal produced a toxicity of the blood, similar to that imparted to the serum by these various procedures *in vitro*, the evidence would be strong. But it is just here that it fails. If the enhancement of the toxicity of serum *in vitro*, by these various substances, really reproduces a process occurring in the blood in the anaphylactic shock, they should be even more effective when injected into the living blood-stream. Experience shows, however, that sols of agar and starch can usually be injected with impunity into a guinea-pig's circulation. Further, if the appearance of toxicity in the serum when treated with these reagents, reproduces the effect of the union of antigen and antibody in the blood, the most uniformly successful method of evoking the toxicity should be to mix serum from an anaphylactic animal with the corresponding antigen. This has been tried a large number of times, and I think it is a fair summary of the results to say that, though successes have been recorded* and emphasized, the usual outcome has been complete failure. The fact that recourse is had to such artificial procedures for preparing the so-called "anaphylatoxin" is sufficient indication of the failure of the natural and obvious method. The rarity with which the anaphylactic serum is rendered toxic by contact with the antigen is in striking contrast with the regularity with which the toxicity is acquired by contact with agar, starch or coagulated protein, which have no power of producing the anaphylactic shock *in vivo*. Still more difficult is it to reconcile these theories of the anaphylactic phenomenon with the fact, already mentioned, that injection of the antigen simultaneously with or soon after the antibody is uniformly without effect, at any rate in guinea-pigs, to the phenomena in which species these theories have been applied. If the shock is due to changes set up in the blood, the optimum conditions should be provided by the simultaneous injection into the blood of the two necessary factors.

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* Richet, Vaughan, *loc. cit.*, Anderson and Frost, 'Hyg. Lab. Bull.,' No. 64, Washington, 1910.

I would not be understood to deny the possibility that liberation of toxic substances may play some part in the anaphylactic shock, or that in some species, in which the phenomena have been less completely analysed, it may play a larger share in the effect than in the guinea-pig. But I do not think that the evidence, in favour of the earlier and simpler view of the nature of anaphylaxis in this species, has been in any way weakened by the immense amount of industry and ingenuity devoted to the search for an anaphylatoxin. In support of the earlier conception, which makes anaphylaxis depend upon a location of the antibody in the cells of vital organs, I propose now to put before you some evidence of a more direct nature, derived from my own experiments.

I may remind you that the most characteristic feature of the anaphylactic shock in the guinea-pig is the contraction of the plain muscle surrounding the bronchioles, causing asphyxiation. It was early shown by Auer and Lewis (*loc. cit.*) that this was independent of the nerve supply, and due to a direct action on the muscle fibres. The only question remaining, therefore, was whether the muscle fibres were themselves sensitive to the antigen, or whether the antigen acted by causing the production of some toxic substance in the blood or in some other organ, which then acted on the plain muscle. I found that, if the lungs of the anaphylactic guinea-pig were removed from the body and their blood-vessels perfused clear of blood by Ringer's saline solution, while the lungs were rhythmically inflated by a pump, addition of a trace of the specific antigen to the perfused fluid caused an immediate constriction of the bronchioles, so intense that air could not be forced past the obstruction. The effect was perfectly specific, and it seemed clear that the action of the antigen on the plain muscle of the bronchioles was direct, and independent of other organs and of the presence of the blood. Other kinds of plain muscle, more convenient for observation when isolated from the body, such as a horn of the uterus suspended in Ringer's solution, gave quite similar results. While my own experiments were in progress, Schultz,* of Washington, published experiments demonstrating the reaction to the antigen of isolated intestinal plain muscle from anaphylactic guinea-pigs. Using the horn of the uterus of a young virgin guinea-pig as an easily isolated and reactive sample of plain muscle, I was able to demonstrate practically all the characteristic phenomena of active and passive anaphylaxis.*

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* 'Journ. Pharmacol. and Exp. Therap.,' vol. 1, p. 549 (1910); vol. 2, p. 221 (1910); 'Hyg. Lab. Bull.,' No. 80, Washington, 1912.

Fig. 1 illustrates the exquisite specificity of the reaction as exhibited by this method. The uterine horn from a guinea-pig rendered sensitive by an injection of $\frac{1}{400}$ c.c. of horse serum, given 14 days previously, was suspended in 250 c.c. of Ringer's solution at 37° C., to which, at A, B, C, D, E, and F, were added in succession doses of 0·1 c.c. of the sera from sheep, cat, rabbit, dog, and man, and of egg-white. None of these substances had any effect. Finally, at G, 0·1 c.c. of horse serum was added, and the uterine muscle at once responded with maximal tonus.

Fig. 2 is taken from a similar experiment, in which the guinea-pig had been made sensitive by an injection of egg-white. 0·5 c.c. of horse serum, at A, is in this case without effect; 0·1 c.c. of egg-white, at B, causes a maximal response.

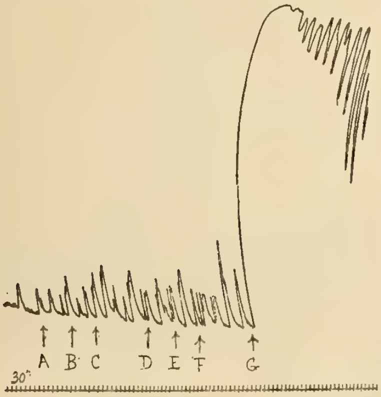

Fig. 1.

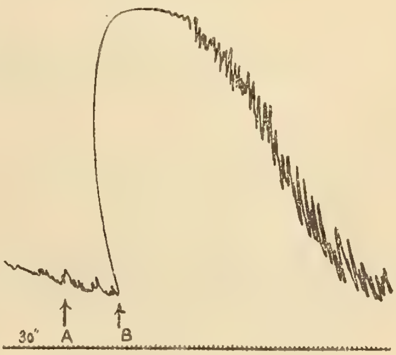

Fig. 2.

Fig. 1 (from 'Journ. Pharmacol. and Exp. Therap.,' vol. 4, p. 214).
Fig. 2 (from 'Journ. Pharmacol. and Exp. Therap.,' vol. 4, p. 180).

Fig. 3 is taken from one of a large number of experiments in which an attempt was made to eliminate the possibility of a participation in the phenomenon by traces of blood or serum left in the vessels of the excised plain muscle. The blood vessels of the uterus were continuously perfused with Ringer's solution for an hour before the organ was removed from the body. The delicacy and specificity of the response are thereby in no degree impaired. The plain muscle, from a guinea-pig sensitive to a horse-serum, is indifferent to cat and sheep serum, added at A and B, but responds maximally to 0·1 c.c. of horse serum at C.

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* 'Journ. Pharmacol. and Exp. Therap.,' vol. 4, p. 167 (1912).

Fig. 4 shows a record of the reaction taken on a faster moving surface. The addition to the bath of Ringer's solution of a small dose of the antigen to which the plain muscle is sensitive, is signalled by a small displacement of the recording lever. The response begins within 10 seconds, with no greater delay than that shown by the response to many drugs similarly applied. It is difficult to suppose that this interval suffices for the elaboration of a poison from the antigen and the body fluids, whether intracellular or extracellular. It suggests rather that the antigen acts as a direct poison on the specifically sensitive cells.

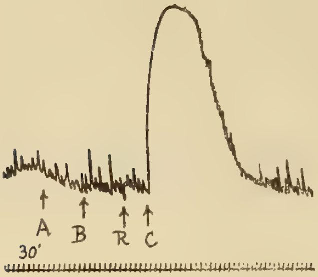

Fig. 3.

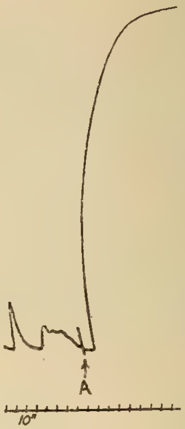

Fig. 4.

Fig. 3 (from 'Journ. Pharmacol. and Exp. Therap.,' vol. 4, p. 177).
Fig. 4 (from 'Journ. Pharmacol. and Exp. Therap.,' vol. 4, p. 178).

It has been suggested that, though a cellular location of the antibody may account for the phenomena of active anaphylaxis, those of passive anaphylaxis must be due to an interaction between antigen and antibody in the circulation. The experiments of Weil, to which I have already referred, served to make it clear that passive anaphylaxis only develops as the transferred antibody leaves the circulation for the tissue cells. Fig. 5, from an experiment on a passively anaphylactic guinea-pig, shows that in this condition also the bloodless plain muscle is specifically sensitive.

The records in figs. 6 and 7 are from a single experiment and show that the sensitive plain muscle, after exposure to an effective dose of the antigen (A), loses its sensitiveness to a further dose (B), but that it can again be rendered sensitive by exposure *in vitro* for some hours to serum from sensitive guinea-pigs. When this is washed away completely by numerous changes of Ringer's solution the plain muscle again responds to the antigen (E), but is again desensitized, and fails to respond to a further dose (F). The dose in each case was 0·05 c.c. of horse-serum.

The uterine plain muscle of a normal guinea-pig can be rendered sensitive by prolonged perfusion with serum from sensitive or immune animals. The sensitiveness is barely perceptible after perfusion for one hour (fig. 8), but clearly developed after five hours (fig. 9). This corresponds with observations on the living guinea-pig, in which sensitiveness cannot be detected until some six hours after antibody from another animal has been injected into the circulation.

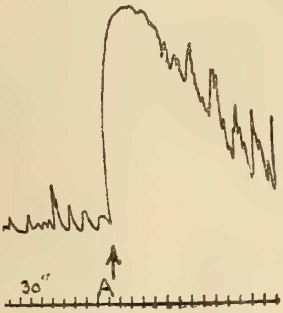

Fig. 5.

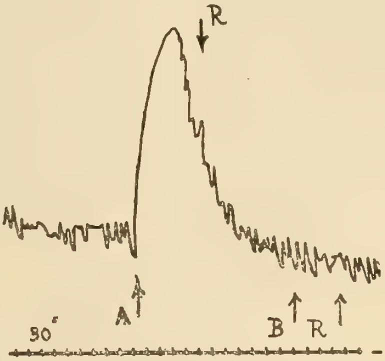

Fig. 6.

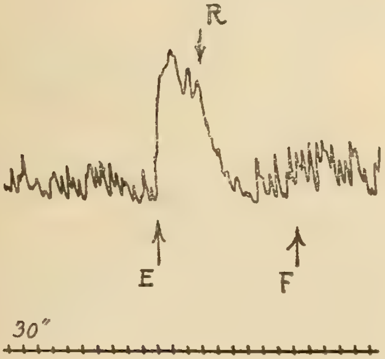

Fig. 7.

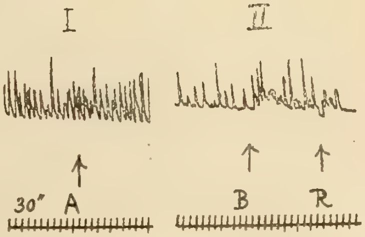

Fig. 8.

Fig. 5 (from 'Journ. Pharmacol. and Exp. Therap.,' vol. 4, p. 196).
Figs. 6 and 7 (from 'Journ. Pharmacol. and Exp. Therap.,' vol. 4, pp. 198, 199).
Fig. 8 (from 'Journ. Pharmacol. and Exp. Therap.,' vol. 4, p. 205).—I. Control horn of normal uterus. II. Second horn of same after perfusion for one hour with 20 per cent. anaphylactic serum. At A and B 0·5 c.c. horse serum.

In the literature of anaphylaxis much confusion has been caused by the use of the inelegant term "antianaphylaxis" for two entirely different conditions. We have seen that the plain muscle exposed to an effective dose of antigen loses its sensitiveness and becomes as indifferent to the antigen as normal muscle. The same phenomenon is seen in the guinea-pig which has recovered from a non-fatal shock; for the time it behaves like a normal animal to further injections, and only slowly reacquires its sensitiveness. In such an animal the reactive antibody has temporarily disappeared. Figs. 6 and 7 illustrate the production of this condition *in vitro*. To this phenomenon of desensitization the name "antianaphylaxis" has been applied. Unfortunately, it has also been used for an entirely different condition. If a guinea-pig is given a series of injections of a foreign protein, spaced so that each follows in succession before anaphylaxis has had time to develop—*e.g.*, at four to five-day intervals—a condition in which very large doses are tolerated is produced, and persists for at least some months. The serum of such an animal contains abundance of the anaphylactic antibody, since it can confer anaphylaxis on a normal animal in much smaller doses than the serum from one which is itself anaphylactic. Nevertheless, the plain muscle of such a truly *immune* guinea-pig, as fig. 10 shows, is as acutely sensitive to the immunising antigen as that from one which is anaphylactic. The immunity is acquired, not by loss of the sensitiveness of the tissues, but by the formation of such excess of antibody that the reaction with antigen is completed in the blood, and none reaches the sensitive cells.

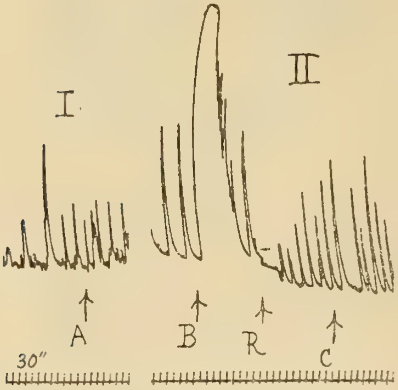

Fig. 9.

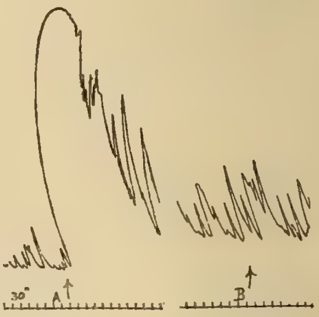

Fig. 10.

Fig. 9 (from 'Journ. Pharmacol. and Exp. Therap.,' vol. 4, p. 206).—I. Control horn of normal uterus. II. Second horn of same after perfusion for five hours with 20 per cent. anaphylactic serum. At A and B 0·5 c.c. horse serum.

Fig. 10 (from 'Journ. Pharmacol. and Exp. Therap.,' vol. 4, p. 194).—Washed plain muscle from guinea-pig rendered *immune* to horse serum. At A 0·5 c.c. horse serum (in 250 c.c.).

These experiments led me, some years ago, definitely to adhere to the theory which regards the condition of anaphylaxis as differing from that of immunity, mainly in the distribution of the specific antibody between the tissue-cells and the circulating fluids, and to regard the anaphylactic symptoms as being caused by the interaction between antigen and antibody occurring in the cells of vital organs. As regards the phenomena seen in the guinea-pig, I believe this view is gradually winning acceptance. In the case of other species the evidence is less complete and the position less clear. Leyton, Leyton, and Sowton,* and also Manwaring,† have maintained that in the rabbit the evidence points to an interaction of antigen and antibody in the blood-stream as the cause of the symptoms of intoxication. In the dog, on the other hand, in which the essential *rôle* of the liver in the anaphylactic reaction had been demonstrated by Manwaring, the experiments of Weil‡ seem to point to a sensitization either of the intrinsic liver cells or of the endothelial cells of liver capillaries, since local introduction of the antigen, into one branch of the portal vein, was found to cause a local reaction, confined to the corresponding lobe of the liver.

Rather, however, than discuss all the evidence as to the nature of the anaphylactic reaction in other species, which would require more time than I have at disposal, I desire to invite your attention to some points of a more general interest, which arise from the conception of the anaphylactic reaction which I have put before you.

We have seen that there is good reason for regarding anaphylaxis as a phase in the production of the type of immunity associated with the precipitin reaction. This represents a mechanism of defence against the incorporation into the tissues of proteins differing in type from those characteristic of the species. Proteins taken in the ordinary way as food are broken in the intestine into their constituent amino-acids, from which the body can again elaborate its characteristic proteins. When an alien protein gains accidental or artificial access to the circulation without undergoing this cleavage, the body slowly forms proteins of which the colloid particles exhibit, with those of the alien protein, the phenomenon of mutual aggregation. If the same foreign protein is again introduced the defensive mechanism is ready, and aggregation occurs at once. It is highly probable that this process is the preliminary to hydrolysis and elimination, for such injection of a foreign protein, into an animal prepared by previous injections, is followed by an increased output of nitrogen in the urine, considerably in excess of that corresponding to the protein injected. If the proteins specially modified to produce this defensive aggregation are located in the cells of vital organs, the animal is said to be anaphylactic, and the sudden reintroduction of the foreign protein may lead to the death of the individual in defence of the type. A discussion of the teleology of the condition would lead us too far into considerations of the pathology of infection. I may content myself with pointing out that the sudden introduction of a foreign protein into the general circulation is not a natural event. An animal may be highly immune to infection with a micro-organism through natural channels, and yet anaphylactic to the proteins of that organism introduced by artificial means. A generalised anaphylactic reaction, the anaphylactic shock, is a creation of the injection needle. A localised anaphylactic reaction, on the other hand, defends the system from invasion at the expense of the tissues immediately affected. Anaphylaxis, as we see it in the laboratory, is not the opposite of immunity; it is the physiological response, of an animal in a certain phase of immunity, to the artificial test which we impose.

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* 'Journ. of Physiol.,' vol. 50, p. 265 (1916).

† 'Trans. Soc. Exp. Biol. and Med.,' vol. 13, p. 173 (1916).

‡ 'Journ. Immun.,' vol. 2, p. 525 (1917).

The most interesting feature of this defensive mechanism is the exquisite fineness of its discrimination between foreign proteins, which by ordinary physical and chemical methods are indistinguishable. All attempts to explain the reactions of immunity on electro-chemical lines, to compare the precipitin-reaction, for example, to the mutual precipitation of two oppositely charged amphoteric colloids, are at least premature, in that they fail, in the present state of knowledge, to account for this phenomenon of specificity. Whatever the difference may be between the proteins which the reaction differentiates, it is nothing so obvious as a difference in the isoelectric point. On the other hand, a mere analysis into amino-acids often shows no difference in composition. Osborne and Wells found that corresponding proteins, from seeds of plants belonging to the same order, *e.g.*, the gliadins from wheat and barley, contained the same amino-acids in apparently the same proportions, though the anaphylactic reaction detected a difference between them. It is obvious, however, that a mere analysis into amino-acids tells very little concerning the structure of the molecule. The vast number of different ways in which an identical large assortment of amino-acids can be linked together offers almost indefinite possibilities of variation in the intimate pattern of the molecular fabric.

The recent work of Dakin and Dudley has given the first hint of a connection, between variation in molecular configuration and the specific difference of corresponding proteins. When a protein is racemised as far as possible by warming with alkali, and then subjected to complete hydrolysis, some of the amino-acids are found to have undergone complete racemisation, while others retain, to a greater or less degree, their original optical activity. Dakin has given reason for believing that the amino-acids which escape racemisation are those occupying the terminal positions in the peptide chains, out of which the large protein molecule is supposed to be built.

Dudley and Woodman* found that the caseins from sheep's and cow's milk, though containing the same amino-acids in the same proportions, showed a small but definite difference when examined by this method. The amino-acids escaping racemisation were not identical in the two cases.

It seemed of interest to determine directly whether a difference of this kind could be related to a difference of behaviour as anaphylactic antigens. Dr. Dakin and I decided to make the comparison on the crystallised albumins from the eggs of the domestic fowl and the duck. Dr. Dakin prepared the two pure proteins and conducted the analysis. The two are indistinguishable by any ordinary method. Their physical properties appear to be identical, and they contain the same amino-acids in apparently identical proportions. The results of the racemisation are shown in Table I. The difference is small but quite definite, and might well be supposed to correspond with a far greater difference in the structure of the whole molecule. What we have here is, as it were, an imperfect glimpse of the edge of the pattern. Figs. 11, 12, and 13 show their behaviour as anaphylactic antigens, the tracings being again records of the contraction of the isolated uterine muscle of specifically sensitised guinea-pigs. Fig. 11 is from a guinea-pig sensitised to hen-albumin. Figs. 12 and 13 are from two horns of the uterus of a guinea-pig sensitised to duck-albumin.

Table I.

Amino-acid.	State of optical activity.		Comments.
	"Racemised" hen-albumin.	"Racemised" duck-albumin.	
Alanine	Not racemised	Not racemised	No difference.
Valine	Partly racemised	Partly racemised	No difference.
Leucine	Mostly racemised	Mostly active	A definite difference.
Proline	Mostly racemised	Mostly racemised	No difference.
Phenylalanine	Completely inactive	Completely inactive	No difference.
Tyrosine	Inactive	Inactive	No difference.
Aspartic acid	Mostly inactive, some active	Completely inactive	Definite difference.
Glutamic acid	Completely inactive	Completely inactive	No difference.
Histidine	Completely inactive	Mostly active	Definite difference.
Arginine	Active	Active	No difference.
Lysine	Inactive	Inactive	No difference.

It must not be assumed that all antigenic difference necessarily depends on this kind of structural difference. There is evidence, for example, in favour of the view that the euglobulin and pseudo-globulin of a serum represent one common basal protein, associated in the one case with a phosphorus-containing lipoid, which confers upon it its distinctive physical properties. Whether this association is also the source of the antigenic disparity can only be decided by a more complete investigation, but that the disparity exists is, I think, beyond doubt. Fig. 14 shows a record of plain muscle from a guinea-pig rendered sensitive by an injection of the euglobulin of horse-serum

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* 'Biochem. Journ.,' vol. 9, p. 97 (1915).

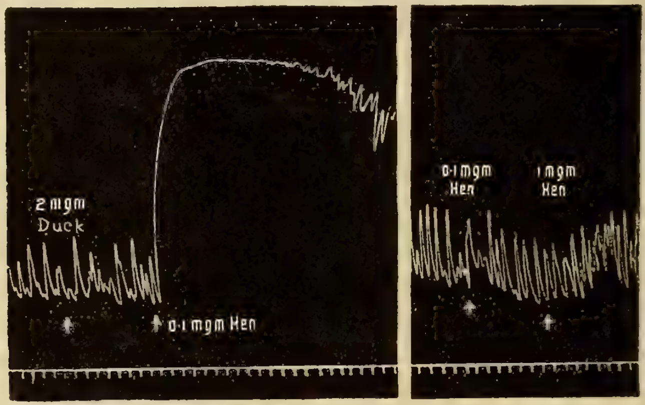

Fig. 11 (from 'Biochem. Journ.,' vol. 13, p. 253, 1919).—Uterus from guinea-pig sensitized by 1 mgrm. of hen egg-albumin. 21st day. Ringer's solution changed at the break in the record. Volume of bath = 70 c.c.

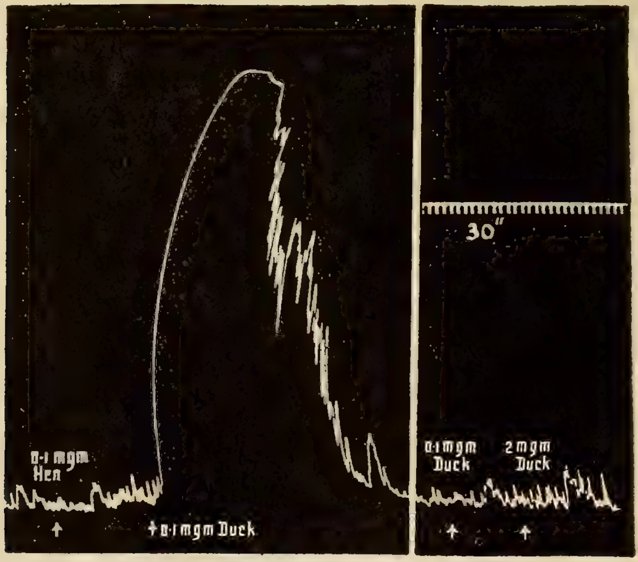

Fig. 12 (from 'Biochem. Journ.,' vol. 13, p. 254, 1919).—Uterine muscle from guinea-pig sensitised by 1 mgrm. of duck egg-albumin, 28th day.

13 days previously. At A and B doses of 1 mgrm. and 10 mgrm. respectively, of the corresponding pseudo-globulin were added to the bath. At C 10 mgrm. of the euglobulin were similarly added, and the same again at D. But the cases of greatest interest, as presenting the greatest difficulty of interpretation, were those in which antigenic difference could not hitherto be related to any chemical or physical difference, but in which an indication of stereochemical difference is now obtained. What may be the nature of the relation between the antibody and the antigen, in what way the antibody is adjusted to form a complex only with a protein of a certain molecular pattern, are points on which I believe no chemist would hazard a suggestion at the present stage. The relation of enzyme to substrate, the symbol of lock and key suggest themselves; but the lock must be much more complicated, the pattern of the key much more intricate than any for which a study of enzymes will provide

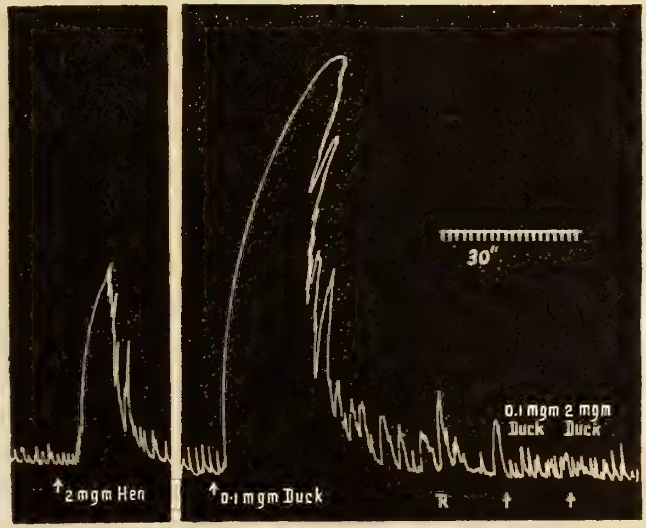

Fig. 13 (from 'Biochem. Journ.,' vol. 13, p. 255, 1919).—Second horn of uterus from same guinea-pig as fig. 12.

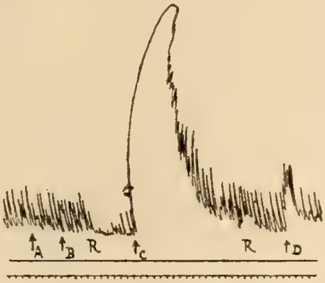

Fig. 14 (from 'Biochem. Journ.,' vol. 10, p. 422, 1916).

analogy. Dakin and Dudley's results seem to constitute a first step towards explanation of what was hitherto a completely mysterious difference. They may even represent a first step, though a very short one, towards a recognition of the chemical differences which underly the characteristics of the species, of the variety, and even of the individual. If a study of anaphylaxis can assist progress towards a solution of this ultimate biological mystery, it will not be difficult to justify a claim for its general biological significance.

There are problems of a more purely physiological nature, to the solution of which I believe that a study of anaphylaxis may contribute. We have seen that the resemblance between the anaphylactic shock and the effects of certain poisons has led to attempts to explain the former in terms of the latter, by assuming that a liberation of poisons of this type is the immediate cause of the shock. This attitude has always seemed to me illogical; it involves the assumption that more is known concerning the mode of action of the poisons than of the antigen on the anaphylactic animal. I believe the reverse to be the case. If, on the evidence as it stands, one phenomenon is to be interpreted by what is known of the other, it is the anaphylactic reaction which must furnish the key to the action of poisons producing the same type of effect, concerning which our ignorance is otherwise complete. Much has been written concerning the action of poisons and their preferential action on certain types of cell. Receptive substances are postulated, on which the sensitiveness of the cell depends. In the case of the anaphylactic cell alone, we are able to obtain a "receptive substance," the antibody, apart from the cell, and to study in the test-tube the nature of its reaction with the "drug," the specific antigen. We are entitled, I think, to suppose that, when antigen meets antibody in the specifically sensitive cell, the immediate effect is a change in the state of dispersion of the colloids entering into its structure. It appears to me to be at least a legitimate working hypothesis to suppose that certain poisonous substances produce similar symptoms because they also bring about a change of this type. We should still have to explain why such a change, occurring in a plain muscle cell, caused it to contract: occurring in an endothelial cell, caused it to become lax and permeable. That explanation would involve a knowledge of the physiological processes of these cells which is not yet available. On the other hand, a knowledge of the nature of the reaction underlying the anaphylactic phenomena may itself contribute to an understanding of the intimate nature of the changes which give rise to contraction and maintain tonus in plain muscle—phenomena which still await their due share in that investigation of the nature of muscular motion, for the promotion of which the Croonian Lecture was founded.

The Relation of Spermatozoa to certain Electrolytes.—II.

By J. Gray, M.A., Fellow of King's College, Cambridge.

(Communicated by Prof. J. Stanley Gardiner, F.R.S. Received October 24, 1919.)

The fact that living spermatozoa move towards the positive pole of an electric field has been known for some years. In a note published in 1915, the writer (8) pointed out that this movement is dependent upon a certain concentration of hydroxyl ions, without which the spermatozoa neither exhibit their normal activity nor do they move in an electric field. In the same paper the behaviour of spermatozoa to such trivalent ions as cerium was briefly described. In the present communication these results are enlarged, and the problem briefly discussed from its theoretical aspect.

A considerable mass of evidence now exists to show that the surface charge of a particle or membrane is profoundly affected by the nature of the solution with which it is in contact. Albumen particles, when suspended in an acid medium, are positively charged; when in an alkaline medium they are negatively charged. Perrin (11) has shown that, if a diaphragm separates two phases between which a potential gradient exists, then the gradient can increase or decrease by treating the diaphragm with various agents. In acid solutions a negative diaphragm becomes less negative, and finally positive; in an alkaline solution the negative charge is increased.

Table I.

Diaphragm.	Solution.	Relative Charge.
Carborundum (negative) ...	0·02M HCl	+ 10
	0·008M HCl	0
	0·002M HCl	− 15
	Water	− 50
	0·0002M KOH	− 60
	0·002M KOH	−105
Naphthalene (negative) ...	0·02M HCl	+38
	0·01 HCl	+39
	0·001 HCl	+28
	0·0002 HCl	+ 3
	0·0002 KOH	−29
	0·001 KOH	−60
	0·02 KOH	−60

It should be noted that the greatest change takes place round the neutral point. Perrin also investigated the effects of lanthanum nitrate upon a carborundum membrane.

Table II.

Conc. of La $(NO_3)_3$.	Relative Charge.
0	−60
0·00004 Mol.	−58
0·0002 „	−18
0·001 „	− 1

These results have been confirmed and enlarged by Mines (9) and 10). This author applied the name of "polarising ions" to those ions which are capable of affecting the surface charge of a membrane or particle to any marked degree. Such ions are the hydrogen ion, hydroxyl ion, and such trivalent ions as cerium, lanthanum, and the citrate ion.

Finally, the work of Chick and Martin (2) on the precipitation of albumen or globulin suspensions by such salts as lanthanum is of great interest. These authors show clearly that the precipitating power of such salts as lanthanum nitrate or sodium citrate depends very closely upon the hydroxyl ion concentration of the colloid suspension. If albumen is dissolved in dilute acid, lanthanum salts do not precipitate the colloid; these salts are, however, powerful precipitants from an alkaline medium. They have also shown that lanthanum is capable of conveying a positive charge to such particles if the salt is sufficiently concentrated. Precipitation only occurs when the "polarising" elements present allow the particles to possess a minimum charge. In other words, precipitation occurs at the isoelectric point.

The application of these principles to physiology was first made by Mines, who investigated the relationship of the vertebrate heart to polarising and other ions. He also showed that red-blood corpuscles suspended in Ringer solution closely resemble the negative particles of an emulsoid colloid.

In 1916 the writer (7) described the effects of lanthanum or cerium upon the electrical conductivity of echinoderm eggs, and reference was made to the possibility that the nature of the surface charge on the egg played an important *rôle* in the phenomena of artificial parthenogenesis. The present experiments with the spermatozoa of *Echinus miliaris* form an attempt to attack the problem of fertilisation from a similar standpoint.

At the outset it is convenient to refer to one or two well-known facts. In the first place, spermatozoa suspended in sea water are surrounded by an alkaline medium whose P_H is about 8·0. In the second place, the interior of the living cell is always more acid than the surrounding medium. Living spermatozoa or eggs stain bright red with neutral red, whereas sea water gives an orange reaction with this indicator. Finally, living spermatozoa migrate to the positive pole of an electric field. We therefore start from the assumption that spermatozoa resemble a suspension of colloid particles in an alkaline medium. It is maintained that the experiments to be described afford strong support for this hypothesis.

The Effects of Trivalent Positive Ions.

A concentration of 0·0005 M $CeCl_3$ or $La(NO_3)_3$ in sea water quickly causes most marked agglutination of a sperm suspension. The spermatozoa are heaped together in irregular masses clearly visible to the naked eye. These masses quickly sink to the bottom of the tube containing the suspension. If the agglutinated spermatozoa are gently centrifuged and washed with normal sea water little or no change takes place in their appearance even after fairly strong agitation.

If, however, to 10 c.c. of sea water containing agglutinated spermatozoa 1 c.c. of 0·8 M* sodium citrate is added, all trace of agglutination rapidly disappears, and the fluid again resembles the original dispersed sperm suspension. The phenomena are very striking with both cerium and sodium citrate solutions. Similar experiments were performed with lanthanum and thorium nitrates.

Table III.

Mol. conc. of cerium.	Degree of flocculation.
0	0
0·00025	+ +
0·0005	+ + +
0·00075	+ + +
0·0010	+ + +
0·00125	+ + +

Note.—In the above and subsequent tables the following symbols will be used:—

- 0 represents a suspension of maximum dispersal.
- + + + represents a suspension of maximum agglutination which quickly settles to the bottom of the fluid.
- + + represents a suspension in which the agglutination is well marked, but settles somewhat slowly.
- + represents a suspension in which agglutination is still visible to the naked eye, but which settles very slowly.
- ⊕ very faint indication of agglutination.

It will be noted that these experiments are an exact parallel to those which can be performed with a suspension of albumen or globulin in slightly

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[*] A solution of this strength depresses the freezing point of water to approximately that of sea water.

alkaline solutions; the phenomena observed are the same in the two cases and are capable of the same explanation.

In the presence of cerium or lanthanum the negative charge on the surface of the cell or the particle is lost and agglutination results. In the presence of citrate, however, the negative charge is restored by the negative trivalent ion, and dispersal consequently takes place.

That the agglutination caused by cerium or lanthanum is completely reversible by sodium citrate is illustrated by the following experiment: 100 c.c. of sperm suspension was agglutinated by means of cerium and the spermatozoa washed in several changes of sea water; 10 c.c. of this agglutinated suspension were placed in several test-tubes to which varying amounts of 0·8 M sodium citrate solution were added. The tubes were *gently* and uniformly shaken:—

Table IV.

Amount of citrate added to 10 c.c. suspension.	P_H.	Agglutination after 15 min.
c.c.		
0	7·9	+ + +
0·5	7·95	+
1·0	8·0	⊕
1·5	8·1	⊕
2·0	8·2	0
2·5	8·3	0
3·0	8·4	0
4·0	8·5	0

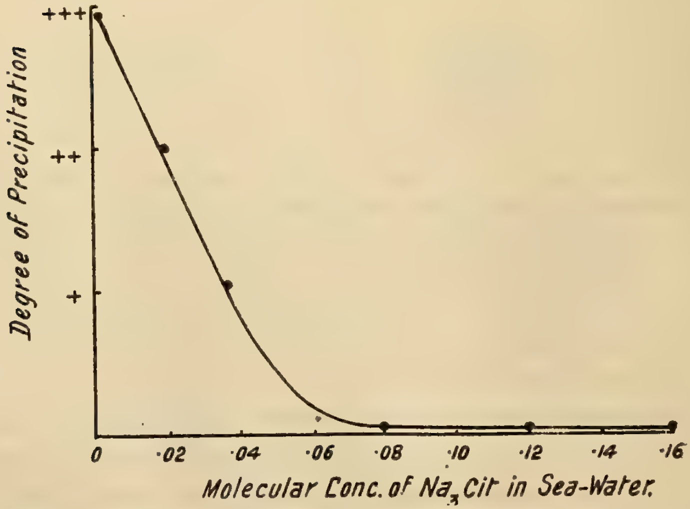

Diagram showing Removal of Cerium Precipitation by Sodium Citrate.

It should be noted that the presence of sodium citrate raises the P_H of the sea water, but this change is insufficient in itself to account for the removal of the effects of the cerium.

That the precipitation of spermatozoa by means of trivalent positive ions depends very closely on the P_H of the solution is shown in the following Table:—

Table V.

P_H of sea water.	Precipitation by 0·0012M $Ce^{\cdots}$.	P_H of sea water.	Precipitation by 0·0012M $Ce^{\cdots}$.
7·6	+ + +	3·7	0
7·3	+ + +	3·3	0
6·5	+ + +	2·9	0
6·1	0⊕*	8·2	+ + +
5·6	0	8·57	+ + +
5·3	0	9·3	+ + +
4·5	0		

* Faintest trace of precipitation.

In different samples of sperm the critical value of P_H varied slightly. In one experiment P_H 7·3 gave no precipitation, in another 6·5 gave none. In each case 7·6 gave strong precipitation.

A number of experiments were performed which brought out the interesting fact that exactly the same hydrogen ion concentration is required to render the spermatozoa motionless as is required to prevent their flocculation by cerium.

Table VI.

20 c.c. sea water + c.c. N/10 HCl.	After 15 min.	
	Movement.	Flocculation by cerium.
c.c.		
0	+ + +	+ + +
0·07	+ + +	+ + +
0·14	+ + +	+ + +
0·21	⊕*	0
0·28	0	0
0·35	0	0
0·42	0	0
0·49	0	0
0·56	0	0

* very slight movement.
+ + + = active movement or complete flocculation.
0 = no movement or no flocculation.

Whereas in normal sea water the cerium produces its effect in less than 30 seconds, in slightly acid sea water the reaction is delayed, and it may be several minutes before incomplete precipitation is apparent.

Three drops of undiluted sperm were placed in 20 c.c. of the following solutions, and after 15 minutes were examined for movement, and tested by cerium (conc. 0·001 M).

In this particular experiment the flocculation in the solution containing 0·14 c.c. N/10 HCl was most marked, and more intense than normal. The experiments illustrate very clearly the intense effects of very small changes of hydrogen ion concentration. A very slight change beyond the critical value stops both movement of spermatozoa and their power of being agglutinated by cerium. Both these phenomena are reversible by raising the P_H of the solution to a value of about 6·5.

In the following experiment the P_H of several samples (20 c.c. each) of the same sperm suspension was lowered to the values shown in column 1; after 20 minutes each sample was divided into two equal portions, and to one portion an equal bulk of sea water was added. To each tube sufficient cerium chloride was added to give a concentration of 0·00125 M $Ce^{\cdots}$. After 15 minutes the degree of agglutination was noted.

Table VII.

I.	II.	I.	II.
P_H of original 20 c.c. of acid suspensions.	P_H of portions of original solution after dilution with sea water.	Degree of agglut. by $Ce^{\cdots}$ in original suspensions.	Degree of agglut. by $Ce^{\cdots}$ in diluted suspension.
7·6	7·7	+ + +	+ + +
7·3	7·5	+ + ⊕	+ + +
6·5	7·3	+ ⊕	+ +
6·2	6·9	⊕	+ +
5·9	6·6	0	+
5·6	6·4	0	+
5·3	6·3	0	+

The reversal of the effect of cerium by sodium citrate, and the dependence of the agglutination by cerium upon the hydroxyl ion concentration of the suspension, are exactly paralleled by the effect of these ions upon a suspension of albumen or globulin. In the latter case, there is no cause to doubt the conclusion that the degree of dispersal or agglutination caused by acids, alkalis, or trivalent ions is dependent upon the surface charge on the particle. The point of maximum flocculation coincides with the reduction of the surface charge to a minimum (in other words, at the isoelectric point); in acid solutions the colloid particle is positively charged so that the positively charged trivalent ions cause no precipitation; in alkaline solutions the particles are negatively charged, and positive trivalent ions cause precipitation by reducing the charge to the isoelectric value.

Finally, it must be mentioned that spermatozoa which have been agglutinated by cerium or lanthanum are dispersed by acid sea water, and the concentration of acid required coincides with the critical values found in such experiments mentioned in Table VII. The addition of further cerium to such acid suspensions causes no agglutination.

This fact is important, as it shows very clearly that the effect of cerium on a normal sperm suspension is of the same nature, but much less intense than that of acids. Acids never agglutinate spermatozoa, but at a critical concentration the surface charge changes with great rapidity from negative to positive, and in each case maximum dispersal exists just as in a weak globulin solution, which contains a high concentration of neutral salts. Cerium or lanthanum, however, have a much less intense action than the hydrogen ion; the trivalent ions reduce the normal negative charge, but they cannot convey a sufficient positive charge to cause dispersion; hence, spermatozoa agglutinated by cerium can be dispersed either by acids or by trivalent negative ions; in the former case dispersion being due to a positive charge and in the latter to a negative charge.

The intense action of the hydrogen ion is paralleled by the experiments of Perrin and Mines, and also by the interesting work of Ellis (4). The latter author has shown that the maximum surface potential on an oil-water interface exists in the region of "neutrality"—the addition of acid very rapidly reduces this potential, while alkalis reduce it somewhat more slowly. This latter point is of interest, when we consider the effects of alkalis upon sperm suspensions. As stated in a previous paper (8), the effect of fairly strong alkaline sea water is to agglutinate the spermatozoa. The action is, however, less complete than that of cerium, and it will be noted that some doubt may exist as to whether the two phenomena are comparable. In the first place, the agglutination of sperm by alkali shows well marked grades of agglutination with increasing $P_H -$, also a concentration of alkali which is sufficient to strongly agglutinate the vast majority of the spermatozoa allows the minority to continue in active movement for a considerable time. Again, with alkali of varying strength, a complete series of intermediate conditions of agglutination is obtained from complete agglutination to complete dispersal, a phenomenon which is not observed in the case of cerium. Finally, concentrations of alkali which cause marked agglutination injure the cells, which then stain yellow instead of red with neutral red, showing that the *interior* of the cell has been affected by the alkali.

It is therefore possible that agglutination of spermatozoa by alkali is a distinct phenomena from that of agglutination by cerium; at the same time it is interesting to note that Ellis found that alkali *reduces* the potential between an oil-water interface, although its action is less intense than that of acids, so that it is possible that the agglutination of spermatozoa by alkali is due to a reduction in the negative surface charge. It should, however, be mentioned that no evidence was obtained to show that the agglutinative power of cerium was increased by raising the P_H of sea water above the normal.

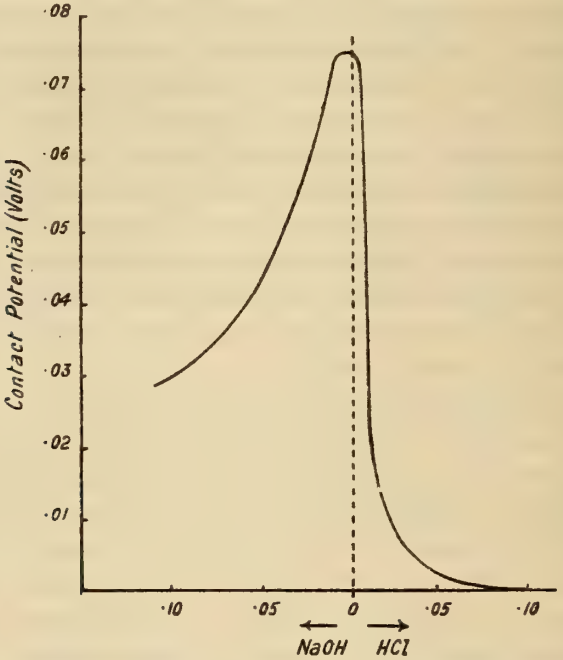

Diagram (after Ellis) showing Relation of Contact Potential of an Oil-Water Interface and Hydrogen Ion Concentration of Water phase.

Summary of Experimental Results.

A suspension of the spermatozoa of *Echinus miliaris* in sea water behaves towards trivalent positive ions in exactly the same way as a suspension of negatively charged particles of such colloids as albumen or globulin. It is only in those solutions which are capable of maintaining the normal negative charge that movement of spermatozoa can take place. Trivalent ions flocculate sperm suspensions by removing the negative charge. The action of the hydrogen ion is very intense and changes the surface charge from negative to positive without any intermediate flocculation.

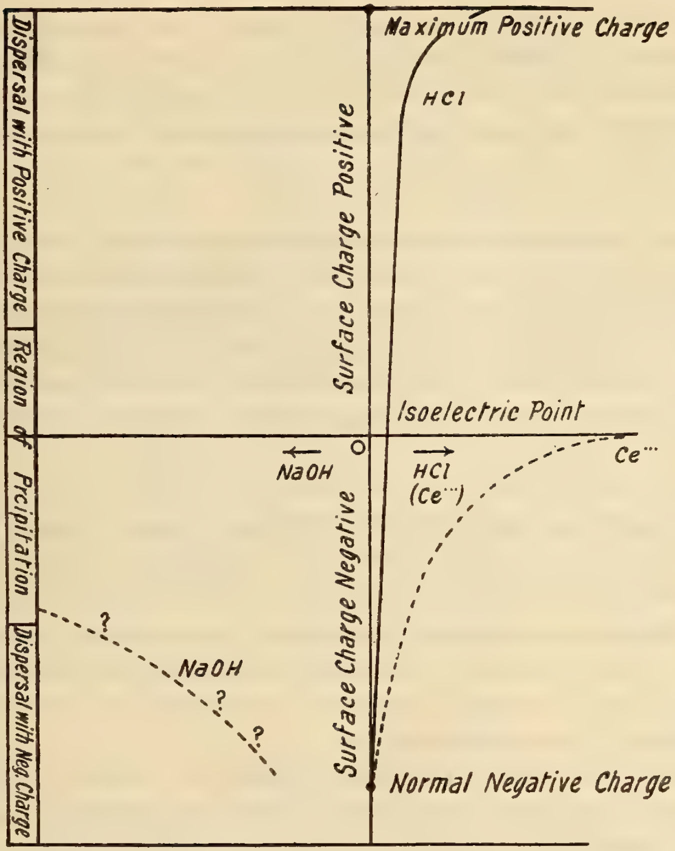
Dispersal with Positive Charge
Region of Precipitation
Dispersal with Neg. Charge
Maximum Positive Charge
HCl
Surface Charge Positive
Isoelectric Point
O
← NaOH
→ HCl ($Ce^{\cdots}$)
$Ce^{\cdots}$
Surface Charge Negative
?
NaOH
?
?
Normal Negative Charge

Diagram illustrating effect of Polarising Ions on Surface Charge and Precipitation of Spermatozoa.

Note on Experimental Hybridisation.

In a previous paper (Gray (7)) it was suggested that the action of various electrolytes upon the eggs of echinoderms was to be found in the action of these substances on the surface charge of the eggs, and that this charge played a fundamental *rôle* in the physiological activity of the egg. In the present paper evidence is brought forward to show that the surface charge on the spermatozoa is of fundamental importance to their activity, and that this charge depends upon the nature of the solutions with which the spermatozoa are in contact. Now, just as the particles of different colloids (or membranes of different composition) possess different charges when in contact with the same solution, so the eggs and spermatozoa of different species may have different surface charges when in sea water of the same composition. If, therefore, the possibility of fertilisation of the egg depends partly on the mutual relationship between the surface charge of the egg and that of the spermatozoon, it is possible that many cases of artificial hybridisation may find a simple solution. One fact is quite certain, namely, that when fertilisation is effected in sea water to which a certain amount of acid has been added, the surface charge of the spermatozoon has been definitely reduced below the normal.

It should be remembered that when both eggs and sperm are exposed to a solution of a definite P_H both gametes will be affected to a certain extent, but the spermatozoa will be very much more affected than the eggs, owing to their much smaller size; also, the egg can react much more strongly to the abnormal conditions, and by an adjustment of its normal metabolism can, conceivably, counteract the effect of an excess of hydrogen ion on its surface charge.*

If eggs of one species and spermatozoa of another are placed in such abnormal conditions, there will be a differential action on the two units, because the surfaces of one species will be altered to a different extent to those of another.

Again, the antagonistic action of mixtures of sperm of two different species may quite likely be due to the changes induced on the surface of the spermatozoa by the changes produced in the hydrogen ion concentration of the suspension by the evolution of CO_2.

In the near future it is proposed to investigate the surface charges of the spermatozoa of different species, with a view to determining whether the possession of a critical surface charge controls the fertilising power of the sperm for eggs of the same and of different species. Since performing the above experiments I have become acquainted with the work of Girard and others (5 and 6) on red blood corpuscles and on bacteria. Girard has shown that very low concentrations of lanthanum decrease the rate at which bacteria migrate towards the anode in an electric field. Further, red blood corpuscles, when suspended in isotonic Ringer solution, behave as negatively charged particles. If, however, they are suspended in acidified saccharose solution, their surface charge is reversed and they become positive. In Ringer solution, however, it is much more difficult to reverse the surface charge by means of acid.

The effect of the hydrogen ion upon the activity of spermatozoa forms the subject of an interesting paper by Cohn (3), which has just come to my notice.

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* The relatively greater sensitivity of spermatozoa to changes in hydroxyl ion concentration has been deduced from other facts by Moore and other workers.

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A Further Study of Chromosome Dimensions.

By C. F. U. Meek.

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(Plates 2 and 3.)

Introduction.

In 1912 I published a paper giving measurements of the chromosomes composing the complexes of species belonging to both closely allied and widely separated divisions of the animal kingdom. The material studied consisted chiefly of the spermatogenetic cell generations : and these investigations, so far as I am aware, constituted the first serious attempt to measure chromosomes and to compare chromosome dimensions throughout the animal kingdom. The results obtained proved certain generalisations, and suggested others.

The first generalisation with which I intend to deal here was that the degree of somatic complexity of an animal cannot be correlated with the lengths of the chromosomes composing its complex. This generalisation was proved by the data collected: for, passing through the various phyla, from simple to highly differentiated animals, no tendency could be found for the chromosomes of the complex to become either longer or shorter; and, outside small divisions of the animal kingdom, our systematic classification gave no clue to the lengths in the complex of any particular animal.

The second generalisation was that the degree of somatic complexity of an animal cannot be correlated with the number of the chromosomes composing its complex. This was likewise proved by the data collected: for no tendency could be found for highly differentiated animals to possess either more or fewer chromosomes than are possessed by low and simple animals; and, outside small divisions, our systematic classification again constituted no index.

The third generalisation was that correlation exists between the degree of somatic complexity of an animal and the total volume of the chromosomes composing its complex. My data showed a tendency for the total volume of chromatin on the spindles to increase as we passed from simple to complex animals, although strict uniformity in this was not observed. This generalisation was therefore suggested by the results obtained.

The fourth and last generalisation was that correlation exists between the degree of somatic complexity of an animal and the diameter of the chromosomes composing its complex. Whereas the lengths of the chromosomes had been found to be arbitrary, the diameters seemed to fall into three definite groups. The diameters in Protozoa were observed to be equal to half those in low Metazoa, while the diameters in low Metazoa were observed to be equal to half those in high Metazoa. This generalisation was therefore suggested by the results obtained, and, since my data showed no exceptions, strict correlation was proposed.

In consideration of the evidence before me, I constructed a working hypothesis. I suggested that the chromatin granules of the simplest Protozoa are converted into rods by linear growth, accompanying evolutionary development and increased complexity of the animal; the rate of growth is not the same in all chromosomes, and rods of various lengths are therefore evolved. A stage in phylogeny is reached later, when chromosomes have attained a maximum length, the limit depending upon physical conditions, possibly connected with spindle mechanism; when this occurs, chromatin units conjugate in fours, and the resulting chromosomes have a diameter equal to twice that previously seen. These then segment into spherical chromosomes of the new diameter, and such chromosomes are prepared to enter a new course of linear growth, accompanying further evolutionary development. When the length limit has again been reached, conjugation of units once more takes place, followed by segmentation into spherical chromosomes of a doubled diameter; and further evolutionary development of the animal is again accompanied by conversion of these spherical chromosomes into rods of various lengths by linear growth.

This hypothesis seemed to accord with the phenomena observed. But the number of animals that I had studied was small, and many phyla had been represented in my investigations by only one species; moreover, the difficulties inherent in the measurement of chromosomes, combined with my inability to eliminate the personal factor from my drawings, increased the possibility of error. I drew attention to this at the time, in order that premature and undue importance might not be attached to the hypothesis, but added the hope that it would be valuable, whether eventually proved or disproved, in that it suggested a new line of thought.

The subject was later studied by Farmer and Digby, who published early in 1914 the results of a series of measurements of chromosomes in both animals and plants. These results partly corroborated and partly disagreed with my earlier observations. On the one hand, they showed a tendency for the chromosome diameter to increase as we passed from simple to complex animals, and further showed that this tendency extended to the plant kingdom, which I had not studied. The similarity shown between the complexes of animals and plants was in many ways remarkable. On the other hand, they proved that the number of different chromosome diameters is greater than I had suspected; that chromosomes composing an individual complex can be of different diameters; and that the chromosome diameters in *Homarus gammarus* are much smaller than those in the closely allied *Palæmon serratus.*

This evidence directly affected my fourth generalisation, previously mentioned; for, while suggesting correlation between chromosome diameter and the degree of somatic complexity, it proved that intimate and uniformly observable correlation could no longer be proposed. In the circumstances, this generalisation became modified accordingly.

In order to obtain further data concerning the third generalisation, and the fourth in its amended form, I turned in the spring of 1914 from spindle measurements to the study of chromosome dimensions in the spermatogenetic mitoses of animals that I had not previously dealt with. I found new and important evidence, particularly in *Smerinthus populi, Vanessa urticæ,* and *Gallus domesticus.* This evidence was supplemented shortly afterwards in a study of these mitoses in Felis, of which preparations were lent to me by Dr. H. de Winiwarter; and I take this opportunity of thanking him again or enabling me thus to study them, and for permitting me to publish photo-micrographs that I made at the time. This work was laid aside in September, 1914, and I have been unable to resume it until this year (1919).

I now intend to give these and other results. The evidence produced will be supported by photo-micrographs in which the differences in chromatin volume and chromosome diameter are distinct enough to be recognised without recourse to actual measurements. These photo-micrographs have been taken from several hundreds that I have made, representing many different animals, and will be found completely to disprove the third and modified fourth generalisations.

Material and Methods.

All material, except that of Felis, was prepared either by me or by my assistant, Mr. T. Russell Goddard.

The testes were preserved in Flemming's strong chromo-aceto-osmic acid fluid, in which they remained for 24 hours. They were then thoroughly washed in running water, and passed through successive strengths of alcohol: and, after being cleared in xylol, were embedded in paraffin. Sections were cut 8μ thick with a Cambridge rocking microtome, and were stained on the slide. The slides were placed for four to six hours in an aqueous solution of ferric alum, and were then stained for 12 to 14 hours in Heidenhain's iron hæmatoxylin.

The preparations were studied with a Zeiss apochromatic oil-immersion objective of 2 mm. focus and N.A. 1·30, and the various compensating oculars. The light was obtained from an inverted incandescent gas lamp, and was passed through a Watson holoscopic oil-immersion substage condenser. All photo-micrographs were made at the same magnification with a Zeiss camera, the objective mentioned above, and compensating ocular No. 4.

Chromatin Volume and Chromosome Diameter.

Figs. 1 to 4 of the Plate represent the grasshopper, *Stenobothrus viridulus.* Fig. 1 shows a polar view of the spermatogonial metaphase. Fig. 2 shows a polar view of the primary spermatocyte metaphase; while fig. 3 shows a lateral view of this metaphase, and the odd or heterotropic chromosome is seen passing undivided to one pole. Fig. 4 shows a polar view of the secondary spermatocyte metaphase. A reference to my first paper and to papers of other cytologists will prove that these photomicrographs are typical both of this species and of other closely allied animals.

Figs. 5 to 11 represent the moth, *Smerinthus populi.* Fig. 5 shows a polar view of the spermatogonial metaphase. Fig. 6 shows lateral views of this metaphase; and a lateral view of the early anaphase is shown in fig. 7.

Figs. 8 and 9 show polar views of the primary spermatocyte metaphase. Fig. 10 shows a lateral view of this metaphase. Fig. 11 shows a polar view of the secondary spermatocyte metaphase. These photo-micrographs closely resemble the drawings of Federley of this and allied species, and may therefore be accepted as typical of these moths.

Figs. 12 to 17 represent the butterfly, *Vanessa urticæ.* Fig. 12 shows a polar view of the spermatogonial metaphase; lateral views of this metaphase are shown in figs. 13 and 14. Fig. 15 shows a lateral view of the primary spermatocyte metaphase. Figs. 16 and 17 show respectively a polar and a lateral view of the secondary spermatocyte metaphase. The similarity of these photo-micrographs and those of *Smerinthus populi* proves that the type depicted is not exceptional among Lepidoptera.

A comparison of the photo-micrographs of *Stenobothrus viridulus* with those of *Smerinthus populi* and *Vanessa urticæ* proves that the total volume of chromatin on the spindle is much greater in Stenobothrus than in either Smerinthus or Vanessa; this difference is equally evident in the spermatogonial, and primary and secondary spermatocyte mitoses. And the same result is obtained from comparison of the chromosome diameters; for, in each cell generation, the diameters in Stenobothrus are obviously much greater than those in Smerinthus and Vanessa. This evidence is similar to that obtained by Farmer and Digby in *Homarus gammarus* and *Palæmon serratus*; but, whereas they observed difference in two animals individually considered, the difference now observed is in animals that have been shown to be typical of definite groups.

Figs. 18 to 23 represent the snail *Helix pomatia.* Fig. 18 shows a polar view of the spermatogonial metaphase. Figs. 19 and 20 show respectively a polar and a lateral view of the primary spermatocyte metaphase. Fig. 21 shows a polar view of the secondary spermatocyte metaphase; while lateral views of this metaphase are shown in figs. 22 and 23. The preparations of Helix studied in my first paper were lent to me, and I stated at the time that I had found few dividing cells, and consequently had been unable to measure all chromosomes of the complex. The photo-micrographs now given are taken from many preparations that I have since made and studied, and I am now able to correct and supplement my earlier observations. I find that the two camera lucida drawings that I gave and identified as spermatogonial mitoses must have been made from spermatocyte cells. Fig. 18 in the Plate of the present paper shows a spermatogonial complex; and the chromosome diameters are seen to be smaller than those observed in the secondary spermatocyte mitosis, represented by figs. 21, 22, and 23. Such difference is not confined to Helix, for I have found it in other animals, including Felis, in which it has been noted by Dr. de Winiwarter. Lastly, the diameters of all chromosomes composing the secondary spermatocyte complex are not the same. Within limits, differences in size of chromosomes seen in a photo-micrograph of a lateral view do not necessarily prove that differences actually exist. All chromosomes of a complex are not in perfect focus at the same moment; and chromosomes that are not in perfect focus appear in the photo-micrograph to be smaller than they really are. In fig. 23, however, certain daughter chromosomes that are equally in focus show small differences in size and diameter; and these differences must, therefore, be accepted. This evidence of different diameters within a complex corroborates the results of Farmer and Digby.

Fig. 24 shows a polar view of the secondary spermatocyte metaphase in the newt, *Triton cristatus*.

Figs. 25 to 29 represent the cat, Felis, and are photo-micrographs that I made from Dr. de Winiwarter's preparations. Fig. 25 shows a polar view of the spermatogonial metaphase, while fig. 26 shows a lateral view of the early anaphase of this mitosis. Fig. 27 shows a polar view of the primary spermatocyte metaphase. Lateral views of this metaphase are shown in figs. 28 and 29, and, in the former, a single chromosome is seen passing apparently undivided to one pole. These photo-micrographs accord with Dr. de Winiwarter's drawings in the plate of his paper. This paper was completed in March, 1914, but its publication has been delayed until this year.

A comparison of these photo-micrographs with those already given shows that the total volume of chromatin on the spindles of Felis is much smaller than that seen in either Stenobothrus or Triton, and the same result is obtained from comparison of the chromosome diameters. But Felis is a mammal and, somatically, is the most highly differentiated animal represented in this Plate. The significance of this evidence has been recognised by Dr. de Winiwarter, who has drawn attention to it in his paper.

Figs. 30 to 36 represent the bird, *Gallus domesticus*. Fig. 30 shows a polar view of the primary spermatocyte metaphase. Figs. 31 and 32 show lateral views of this metaphase. Figs. 33 and 34 show polar views of the secondary spermatocyte metaphase; and lateral views are shown in figs. 35 and 36. These photo-micrographs clearly prove that the total volume of chromatin on the spindles is much smaller than that observed in the corresponding mitoses of Stenobothrus and Triton. Unfortunately, the chromosomes are crowded in the equatorial plane of the spindles, and cannot be measured individually; but the photo-micrographs of lateral views prove that the chromosome diameter cannot be as great as that seen in Stenobothrus and Triton.

The evidence that we have now considered is remarkable. We have observed that the total volume of the chromatin on the spindles may be much smaller in a mammal and a bird than in a grasshopper; while a very great difference was seen between a grasshopper, on the one hand, and a moth and a butterfly on the other—animals that not only belong to, but have been shown to be typical of closely allied divisions of the animal kingdom. And the same results have been obtained in the case of the chromosome diameter. The animals dealt with belong to various divisions of the Arthropoda, Mollusca, and Vertebrata; but a comparison of the photo-micrographs given in the Plate completely fails to show correpondence between our systematic classification and a classification based upon either chromatin volume or chromosome diameter. In the circumstances, we must realise that the tendencies observed in our earlier data for the chromatin volume and chromosome diameter to increase as we passed from simple to complex animals must have been fortuitous. The evidence now before us proves conclusively that the suggestions embodied in my third generalisation and in my fourth in its amended form must be abandoned.

Conclusion.

The evidence produced in my first paper proved two negative generalisations, viz., that the degree of somatic complexity of an animal cannot be correlated with either the lengths or the number of the chromosomes composing its complex. Our new evidence has proved two more negative generalisations, viz., that the degree of somatic complexity of an animal cannot be correlated with either the total volume or the diameters of the chromosomes composing its complex. Cytometrical investigations have failed so far to give a positive generalisation concerning these phenomena. But the work done has been justified; for many facts have been discovered, and the establishment of these four negative generalisations must reduce the number of possible explanations.

If now we consider the hypothesis that I put forward, we find that the new evidence necessitates the elimination from it of all reference to connection between increase of somatic differentiation, resulting from evolutionary development, and increase of chromatin volume and chromosome diameter. The hypothesis thus becomes nothing more than a suggested explanation of the process by which increase in chromosome length and diameter is effected. But the discovery of many different chromosome diameters removes the grounds for suggesting that increase in diameter is discontinuous and is effected by conjugation of units, while increase in length is continuous and is effected by growth. These increases may occur in a way similar to that suggested; on the other hand, the process may be entirely different.

That the complex of a species is a constant seems more and more evident; and, within small groups of the animal kingdom, we can foretell the approximate composition of the complex in a particular species. This has led to the supposition that, throughout the animal kingdom, a classification based upon chromosomes will be found to correspond with the classification based upon somatic characters. That this is not so has been clearly demonstrated by comparison of the photo-micrographs given in this paper. Our studies of chromosomes have failed completely to discover the factors determining the composition of the complex.

The phenomena of the cell must be intimately connected. The evolution of the spindle cannot be dissociated from that of the chromosomes; and elucidation of the one problem will lead probably to elucidation of the other. How and when elucidation will be found cannot be prophesied. Both problems are important; and both are difficult.

Summary.

1. The degree of somatic complexity of an animal cannot be correlated with the lengths of the chromosomes composing its complex.
2. The degree of somatic complexity of an animal cannot be correlated with the diameters of the chromosomes composing its complex.
3. The degree of somatic complexity of an animal cannot be correlated with the total volume of the chromosomes composing its complex.
4. The degree of somatic complexity of an animal cannot be correlated with the number of the chromosomes composing its complex.
5. There are many different chromosome diameters.
6. The chromosomes composing the spermatogonial complex of an animal are not necessarily identical in diameter with those composing its secondary spermatocyte complex.
7. All chromosomes composing an individual complex are not necessarily of the same diameter.

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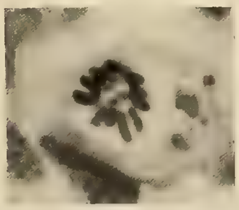
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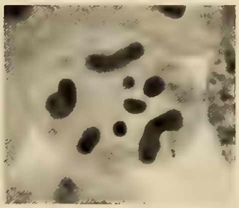
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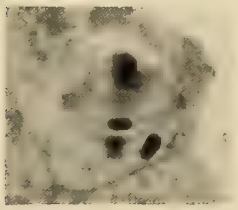
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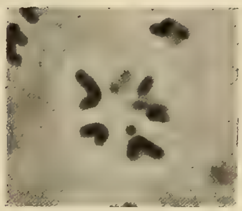
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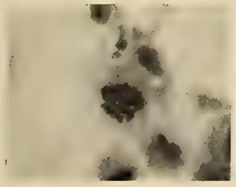
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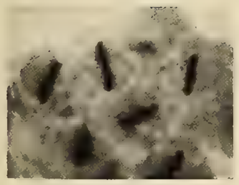
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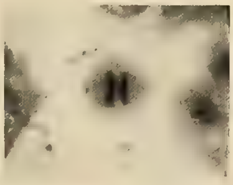
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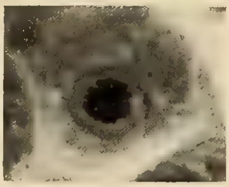
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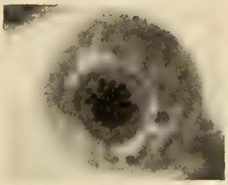
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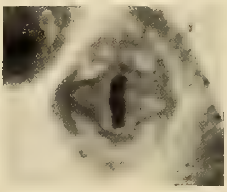
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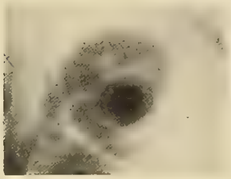
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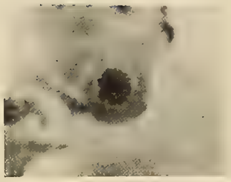
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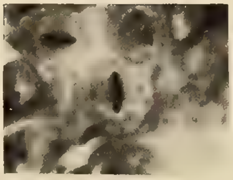
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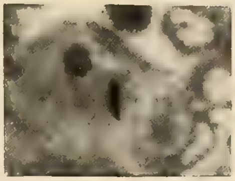
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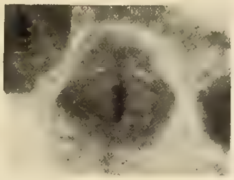
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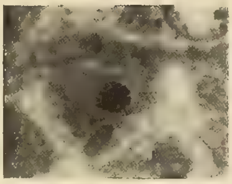
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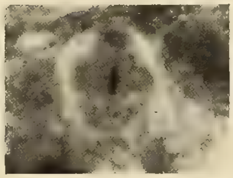
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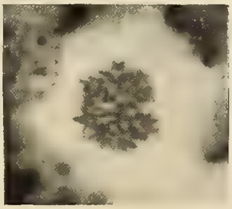
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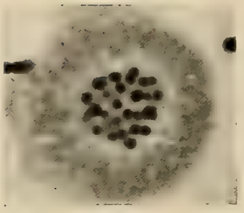
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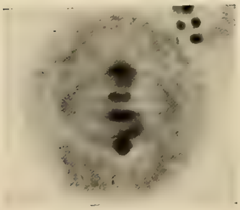
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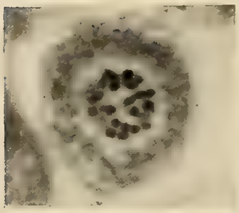
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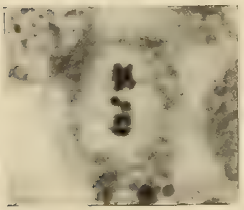
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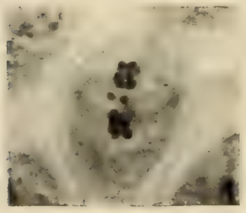
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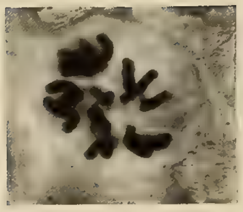
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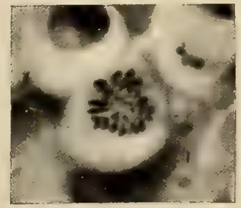
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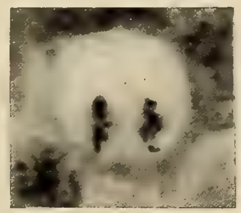
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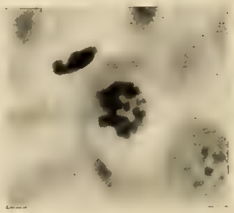
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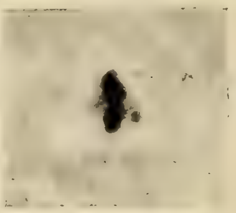
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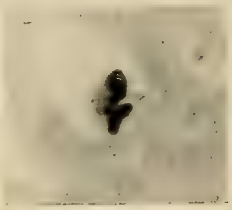
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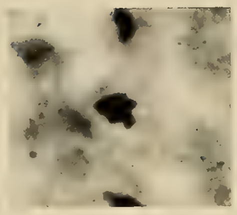
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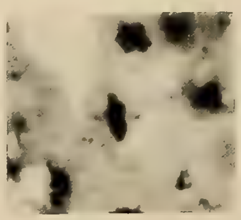
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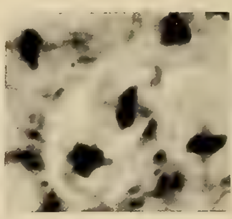
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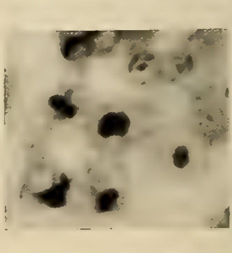
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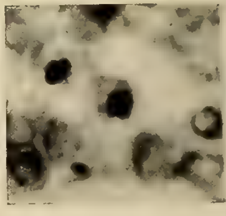
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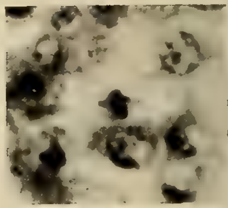
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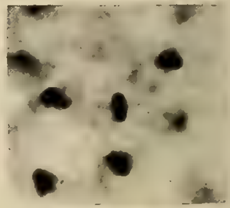
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EXPLANATION OF THE PLATES.

Plate 2.

Fig. 1.—Polar view of spermatogonial metaphase in *Stenobothrus viridulus.*

Fig. 2.—Polar view of primary spermatocyte metaphase in ditto.

Fig. 3.—Lateral view of primary spermatocyte metaphase in ditto, showing the odd or heterotropic chromosome passing undivided to one pole.

Fig. 4.—Polar view of secondary spermatocyte metaphase in ditto.

Fig. 5.—Polar view of spermatogonial metaphase in *Smerinthus populi.*

Fig. 6.—Lateral views of spermatogonial metaphase in ditto.

Fig. 7.—Lateral view of spermatogonial anaphase in ditto.

Figs. 8 and 9.—Polar views of primary spermatocyte metaphase in ditto.

Fig. 10.—Lateral view of primary spermatocyte metaphase in ditto.

Fig. 11.—Polar view of secondary spermatocyte metaphase in ditto.

Fig. 12.—Polar view of spermatogonial metaphase in *Vanessa urticæ.*

Figs. 13 and 14.—Lateral views of spermatogonial metaphase in ditto.

Fig. 15.—Lateral view of primary spermatocyte metaphase in ditto.

Fig. 16.—Polar view of secondary spermatocyte metaphase in ditto.

Fig. 17.—Lateral view of secondary spermatocyte metaphase in ditto.

Fig. 18.—Polar view of spermatogonial metaphase in *Helix pomatia.*

Plate 3.

Fig. 19.—Polar view of primary spermatocyte metaphase in ditto.

Fig. 20.—Lateral view of primary spermatocyte metaphase in ditto.

Fig. 21.—Polar view of secondary spermatocyte metaphase in ditto.

Figs. 22 and 23.—Lateral views of secondary spermatocyte metaphase in ditto.

Fig. 24.—Polar view of secondary spermatocyte metaphase in *Triton cristatus.*

Fig. 25.—Polar view of spermatogonial metaphase in *Felis.*

Fig. 26.—Lateral view of spermatogonial anaphase in ditto.

Fig. 27.—Polar view of primary spermatocyte metaphase in ditto.

Figs. 28 and 29.—Lateral views of primary spermatocyte metaphase in ditto; in fig. 28 a chromosome is seen passing apparently undivided to one pole.

Fig. 30.—Polar view of primary spermatocyte metaphase in *Gallus domesticus.*

Figs. 31 and 32.—Lateral views of primary spermatocyte metaphase in ditto.

Figs. 33 and 34.—Polar views of secondary spermatocyte metaphase in ditto.

Figs. 35 and 36.—Lateral views of secondary spermatocyte metaphase in ditto.

The Physiological Cost of Muscular Work Measured by the Discharge of Carbon Dioxide. Part I.—*The Energy Output of Dock Labourers during "Heavy Work."*

[Interim Report to the Royal Society Food (War) Committee.]

By A. D. Waller, M.D., F.R.S.

(Received November 22, 1919.)

The metabolism Sub-Committee of the Food (War) Committee of the Royal Society at its first meeting considered the following two methods of inquiry for the determination of the energy output of men and women workers :—

A. The Douglas-Haldane method, by which determinations of CO_2 and of O_2 are made, was adopted for recommendation to new workers as the standard method.

B. Waller's method by which determinations of CO_2 alone are made at short frequent intervals, was to be taken on trial.

Allocations for the necessary expenses were made, and interim reports, dealing with the results obtained by both methods, were presented to and considered by the Sub-Committee at several subsequent meetings. Of these interim reports, two dealing with results of method A have recently been communicated to and published* in the 'Proceedings of the Royal Society,' and the present interim report of method B is submitted for similar publication. It deals with the CO_2 output of heavy workers, for whom the energy output has been estimated by the Food (War) Committee at 1100 to 2000 calories per 8 hours per "average man" [*i.e.*, 78 to 142 calories per square metre per hour].

Both methods A and B afford the measure of energy output by readings of the respiratory exchange. By method A the intake of oxygen and the output of carbon dioxide are measured; by method B the output of CO_2 alone.

By method A the relation quotient CO_2 plus/O_2 minus is ascertained, and its variation taken into the calculation of the final energy value of the exchange, which is expressed in calories per square metre.†

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[*] O. Rosenheim, "A Preliminary Study of the Energy Expenditure and Food Requirements of Women Workers," 'Roy. Soc. Proc.,' No. 635, B, vol. 91, p. 44; M. Greenwood, C. Hodson, and A. E. Tebb, "Report on the Metabolism of Female Munition Workers," *ibid.*, p. 62.

[†] In accordance with the practice of the Carnegie Laboratory. A full account of the method is given by Cathcart in the 'Journal of the Royal Army Medical Corps,' November, 1918.

In method B, the results are directly read and expressed in terms of CO_2 output as cubic centimetres of CO_2 per second. The final energy value if required is calculated on the assumption of a normal value of the respiratory quotient $CO_2/O_2 = 0{\cdot}85 \pm 0{\cdot}05$. It is claimed that the range of error involved by A can be reduced to ± 1 per cent., in method B to ± 5 per cent. The speed with which useful data can be obtained is much greater by method B than by method A.*

Errors of Observation.

Errors of timing, of volume reading, corrections for temperature and pressure, errors of CO_2 (and O_2) readings, and clerical errors of arithmetic can occur by both methods; their detailed consideration would occupy too much space, and must be deferred. I shall assume that due care and accuracy have been given to the carrying out of both methods, and consider in detail only the error due to the assumption of a normal respiratory quotient which forms the essential difference between methods A and B.

In the first place it should be realised as being not an error of observation peculiar to method B, but an error of translation, occurring when the results measured in terms of cubic centimetres CO_2 per second are converted into calories—calories per hour, or calories per square metre per hour.

This error of translation can be evaluated from the following two considerations:

1. Its maximal (improbable) value with maximal (improbable) fluctuations of the CO_2/O_2 quotient, *e.g.*, between 0·7 and 1·0 which are its theoretical values on diets exclusively of fat or exclusively of carbohydrates.

2. Its practical (probable)† value with practical (probable) fluctuations of the CO_2/O_2 quotient.

1. Its maximal value is to be appreciated from the following Table of Equivalents of 1 c.c. CO_2:—

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* With Method B, Miss De Decker's ordinary day's work on dock labourers was, for Perry, 20 readings per day (10 double readings), for King and others only 10 per day. But in the laboratory when getting out CO_2 curves, she has worked up to 80 per day. None of her hourly readings on labourers has been rejected or amended. The net expenditure of time at the docks and at Smithfield was 27 days and 6 nights, and the data collected have not involved the employment of an expert computer. By Method B, Greenwood obtained a total number of 226 readings during the period November, 1918, to March, 1919, upon 43 subjects engaged upon various kinds of work.

† Not probable error in the technical statistical sense. My data are not numerous enough for calculation of "probable error."

Respiratory quotient CO_2/O_2.	Calories.	Kilogrammetres.	1 c.c. CO_2 per sec. signifies.
1·00	5·047	2·155	18·17 Kals per hour.*
0·95	5·317	2·270	19·14 " "
0·90	5·587	2·386	20·11 " "
0·85	5·856	2·501	21·08 " "
0·80	6·126	2·615	22·06 " "
0·75	6·396	2·731	23·02 " "
0·70	6·667	2·846	24·01 " "

* To reduce clerical mistakes, I am accustomed to write "cal" for the small or gramme calorie, and "Kal" for the large or kilogramme calorie.

by inspection of which it appears:

(1) That the maximum error between the extremes 1·00 and 0·70 is ±13·79 per cent.

(2) That the practical error between the probable range 0·90 and 0·80 is ±4·598 per cent.

I have allowed therefore as inherent to method B the practical (probable) error = ±5 per cent.

There is an error of observation inherent to short (1 to 5 min.) samples that requires particular consideration at this stage.

In forced breathing, either voluntary without other muscular exertion, or involuntary in consequence of work, there is increased ventilation and increased output of CO_2, which is pumped out of the pulmonary alveoli and blood to an exaggerated degree. It is difficult in a short sample taken during increased work to assign the shares of increased muscular action and of increased pump action in the total increase exhibited by a short sample, which therefore cannot be regarded as a fair sample indicative of increased metabolism. The objection is well founded as regards brief sampling during the period of establishment of augmented breathing, but not as regards brief sampling when the augmented *régime* has become established. During the first minute or two of increased muscular activity the increased expiration of CO_2 is partly due to increased pumping action, partly to increased muscular metabolism; but at the end of five minutes (or less) of steady work a steady *régime* of CO_2 output is established, during which the rate of output is unaffected, or inconsiderably affected by variations of pump effect. A person breathing at rest 6 litres per minute at 3 per cent. CO_2, *i.e.*, discharging CO_2 at the rate of 180 c.c. per minute or 3 c.c. per second, on commencing work at say 10 kgrm. per second exhibits at once an increased ventilation, accompanied by a rise of CO_2 percentage and a total increased output of CO_2, which during the first minute of effort is certainly the resultant of two factors, increased pumping out of CO_2 by the lungs and increased muscular metabolism; but as time passes, the CO_2 output reaches a maximum, and a constant *régime* is established, during which the CO_2 ordinate serves as a true physiological indicator of muscular metabolism. This constant *régime* is reached very rapidly, *i.e.*, in a very few minutes—certainly in five minutes, sometimes even less—and while it lasts, the CO_2 ordinate is a faithful indicator of the internal physiological activity. During the constant *régime* of deeper breathing caused by work, the physiological order of events is (1) increased production of CO_2 causing (2) greater thoracic movements by which the increase is got rid of. With the increased ventilation there is of course increased pump action, and both these factors are equally physiological, in as much as both are excited by the state which they serve to relieve.

And so the muscular metabolism of a workman at steady work—preferably piece-work—is usefully measured by short samples of expired air from which his "CO_2 ordinate," *i.e.*, his established rate of CO_2 discharge, is ascertained. The procedure has two principal advantages: (1) The interruption of work is trifling; it is, *e.g.*, cheerfully tolerated by piece-workers, to whom time is money; (2) The sampling is taken under actual conditions of work, and as nearly as possible during work. Work done in a respiration chamber, or for a few minutes for the sake of affording a sample, cannot be regarded as normal or as affording a fair sample of the respiratory activity obtaining during normal work.

As is shown in this preliminary report, the constant *régime* during work is established at the beginning of work and disestablished at the end of work with surprising rapidity. Samples should not be taken during either of these periods of change. They should be taken if possible without interruption of work in progress, or if this should be impossible, with least interruption for the shortest practicable periods—20 to 30 seconds—immediately after the load or tool has been dropped as, *e.g.*, in the carrying of coal or grain or meat, which form the hardest labour going on in the London docks. An obvious drawback to short sampling is, of course, the possible error arising from turning the tap at beginning and end of say 30 seconds during different phases of respiration. This error is to be avoided by turning the tap on and off at the same phase, *i.e.*, immediately after expiration. In the mouthpiece which I prefer, the expiratory valve click is easy to follow, and I have not found any disturbance of the subject to be caused by the valve clicking, as has been reported by some observers. For a subject breathing say 15 per minute, it is 2 to 1 against the tap being closed during the expiratory movement; but if this should happen, a considerable error, amounting to perhaps 10 per cent., might be made in a half-minute sample.

First Observation (*Tuesday, December* 3*rd,* 1918).

Labourer No. 1, age 51, weight 84 kgrm., height 1·71 metre.

A regular labourer (building and concrete laying) at the East Surrey Docks. Work begins at 7.30 A.M., ends at 4.30 P.M., with dinner hour from 12 to 1, during which he walks ¼ mile home and back.

The work in progress was of varied character consisting in the preparation for the laying of a concrete floor—carrying tools and materials, shovelling and wheeling gravel, mixing concrete, spreading, levelling, "tapping" and "trowelling."

Weight of tapper 12 to 14 lb., height of lift about 18 in., 20 to 25 blows per minute.

"Tapping" is considered by the men as heavy work, but does not amount to more than 1 kgrm.-metre per second.

Time.	Time of collection, secs.	Ventilation.		CO_2.	
		Litres.	C.c. per sec.	Per cent.	C.c. per sec.
Luncheon hour 12–1	—	—	—	—	—
1 h. 0 m.	37	14	378	2·0	7·56*
2 m.	35	13	354	2·0	7·08*
2 h. 0 m.	30	14	446	3·0	13·98
2 m.	30	11	366	2·2	7·32
2 h. 30 m.	30	15	500	3·0	15·00
32 m.	60	10	333	2·6	8·65
3 h. 0 m.	16	16	533	3·4	18·12
3 m.	12	12	200	2·9	5·80

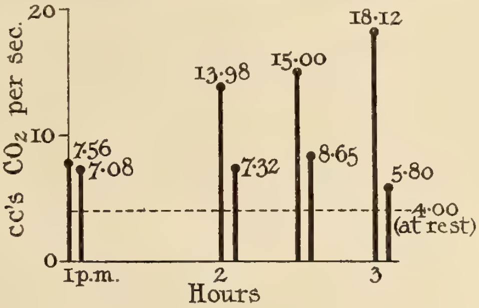
* *Régime* not established.

This was an orientation observation, by which the system of sample collection was shown to the labourer for the first time. No difficulty in the use of the apparatus was experienced and no serious interruption of work occurred. Serviceable data were obtained at once. The effect of a brief rest for two or three minutes was evident, also the gradually increasing output of CO_2 during the afternoon. Three last readings were 13·98, 15·00, and 18·12 c.c. CO_2 per second, *i.e.*, an average output of 15·7 c.c. (gross) per second (= 319·7 Kalories per hour).

I quote this first observation in order to show that the collection of "first observations on a reasonably large" number of subjects under their normal conditions of work is a legitimate task and likely to afford serviceable data.

From inspection of the work in progress it appeared to me that the operation known as "tapping" (in which a known weight is lifted to a regular height at a regular rhythm) is one that lends itself best to a direct mechanical estimation. But the detailed study of this particular form of work was foreign to my present purpose, which is to make good the method of estimating the cost of work of unknown mechanical equivalence in terms of CO_2 discharged.

The next step in the enquiry was to take hourly observations of the CO_2 discharge for the complete day's work and for several days.



Labourer No. 1.—Age 51. Weight (clothed)* 84 kilos.; height 1·71 M. (Surf. 1·96 M^2.) Wednesday, December 4, 1918.

Time.	Time of collection, secs.	Litres.	C.c. per sec.	CO_2 per cent.	CO_2 c.c. per sec.	Remarks.
7 h. 30 m.	60	11	183	2·4	4·39	After ¼ hour walk followed by 5 min. sitting.
8 h. 30 m.	45	12	266	2·8	7·48	Laying concrete floor. "Trowelling" and "tapping."
33 m.	60	13	216	2·3	4·96	
9 h. 30 m.	42	14	333	3·2	10·65	
34 m.	60	12	200	2·8	5·60	
10 h. 30 m.	40	17	425	3·5	14·86	"Tapping."
34 m.	60	14	233	3·0	7·00	
11 h. 30 m.	45	20	444	3·4	15·09	Trowelling on his knees.
34 m.	60	11	183	2·8	5·12	
1 h. 0 m.	55	15	272	3·0	8·16	Immediately after ¼ mile walk, fast.
2 h. 0 m.	43	18	418	2·8	11·70	"Trowelling" and "tapping."
3 m.	60	14	233	2·2	5·12	
3 h. 0 m.	45	20	444	3·2	14·20	
4 m.	60	17	283	2·4†	6·79	
4 h. 0 m.	43	21	488	3·8	18·54	
4 m.	60	18	300	2·6	7·80	

* Four kilos. allowed for weight of clothes.

† Sample analysed by small Haldane apparatus CO_2 = 2·5 per cent. O_2 = 2·3 ,, ,, R.Q. = 1·09.

The points of note in this observation are:—

1. The hourly increase of CO_2 discharge during each period of work.
2. The restorative effect of the dinner-hour.
3. The rapid fall of CO_2 during a rest pause of three or four minutes,

showing that it is necessary to take samples with least possible delay. The average discharge for the last three hours of forenoon was 13·53: of afternoon 14·78.

4. The maximum discharge is greater at the end of the afternoon than of the forenoon.

The volume of ventilation and the percentage of CO_2 were both higher in the first sample, taken immediately after the labourer had dropped tools than in the second sample, taken after a pause of three minutes.

There is, as may be seen throughout the protocols, a general correspondence between volume of ventilation and amount of CO_2 per second, so that it is allowable, with due reservation, to make use of ventilation alone as a guide to work done. I have found this guide of value in prolonged observations, and when it was not possible to take samples by bag quickly enough to follow the change of output of CO_2 that takes place at the beginning and end of a spell of work. For this purpose I have adapted a Verdin spirometer as a litre recorder of expired air during rest and during certain kinds of work. The results obtainable by this means will be reported upon later.

Labourer No. 1.—Thursday, December 5, 1918.

Time.	Time of collection in secs.	Litres.	C.c. per sec.	CO_2 per cent.	CO_2 c.c. per sec.	Remarks.
7 h. 30 m.	60	12	200	2·5	5·0	After ¼ mile walk followed by 5 min. rest.
9 h. 0 m.	40	22	555	2·8	15·54	Trowelling and tapping.
3 m.	60	23	383	1·7	6·51	
10 h. 0 m.	42	21	500	3·0	15·00	Moving tools to another part of the building.
3 m.	60	18	300	2·8	8·40	
11 h. 0 m.	40	22	550	3·1	17·05	Moving gravel, etc., and other building materials.
3 m.	60	17	233	2·9	6·75	
11 h. 45 m.	40	23	575	3·6	20·70	
48 m.	60	19	316	3·0	9·48	
1 h. 0 m.	60	18	300	2·8	8·40	After ½ mile fast walk.
2 h. 0 m.	40	21·5	537	3·0	16·11	Work rather heavier than usual.
3 m.	60	20	333	2·6	8·65	
3 h. 0 m.	40	23	575	3·5	20·12	
5 m.	60	19	316	3·2	10·11	
4 h. 0 m.	40	22	550	3·5	19·80	Trowelling floor.
4 h. 3 m.	60	10	333	3·0	10·00	

The time of collection of samples was chosen as one minute when the subject was at rest, but when he was at work it was necessary to collect for a shorter period; the beginning and end of each period was made by sharply turning the tap at the end of an expiration, as indicated by the valve click. No disturbance by reason of this click was experienced by any of the subjects; it served to count the respirations, which, however, were of such constant frequency under conditions of work and rest that no attempt was made to take systematic frequency counts.

Points of Note.—The hourly increase of CO_2 discharge during the morning and the afternoon; average discharge for last three morning hours = 17·58, for the afternoon hours 18·67; the restorative effect of the dinner hour and of short rests.

Labourer No. 1.—Friday, December 6, 1918.

Time.	Time of collection in secs.	Litres.	C.c. per sec.	CO_2 per cent.	CO_2 c.c. per sec.	Remarks.
7 h. 30 m.	60	14	233	2·2	5·12	After ¼ mile walk and 10 min. rest.
8 h. 0 m.	50	22	440	2·8	12·32	Tapping and trowelling.
3 m.	60	18	300	2·4	7·20	
9 h. 0 m.	40	21	525	2·8	14·70	
3 m.	60	20	333	2·5	8·32	
10 h. 0 m.	40	22	555	3·0	16·65	
3 m.	60	18	300	3·0	9·00	
11 h. 0 m.	40	24	600	2·9	17·40	
3 m.	60	20	333	2·7	9·00	
12 h. 0 m.	40	23	575	3·0	17·00	
3 m.	60	19	316	2·8	8·80	
1 h. 0 m.	60	21	350	2·8	9·8	Immediately after ¼ mile walk.
2 h. 0 m.	45	22	488	3·0	14·64	Tapping and trowelling.
3 m.	60	19	316	2·7	8·53	
3 h. 0 m.	40	20	500	3·3*	16·50	
3 m.	60	16	266	3·1*	8·24	
4 h. 0 m.	40	23	575	3·4	19·50	
3 m.	60	20	333	3·0	10·00	

* Two samples of each of these two bags were collected at once by mercury displacement and handed over to Dr. Pembrey for independent analysis. His report was as follows :—

	CO_2.	O_2.	R.Q.
Sample I	3·16	2·96	1·04
Sample II	2·99	2·97	1·00

The noteworthy points are as on the first two days, viz., the progressive hourly increase of CO_2, and the restorative effect of the dinner hour and of short rests.

Average hourly discharge of CO_2: forenoon, 17·02; afternoon, 16·88.

The mean hourly discharge of CO_2 (gross expenditure) for the three days was :—

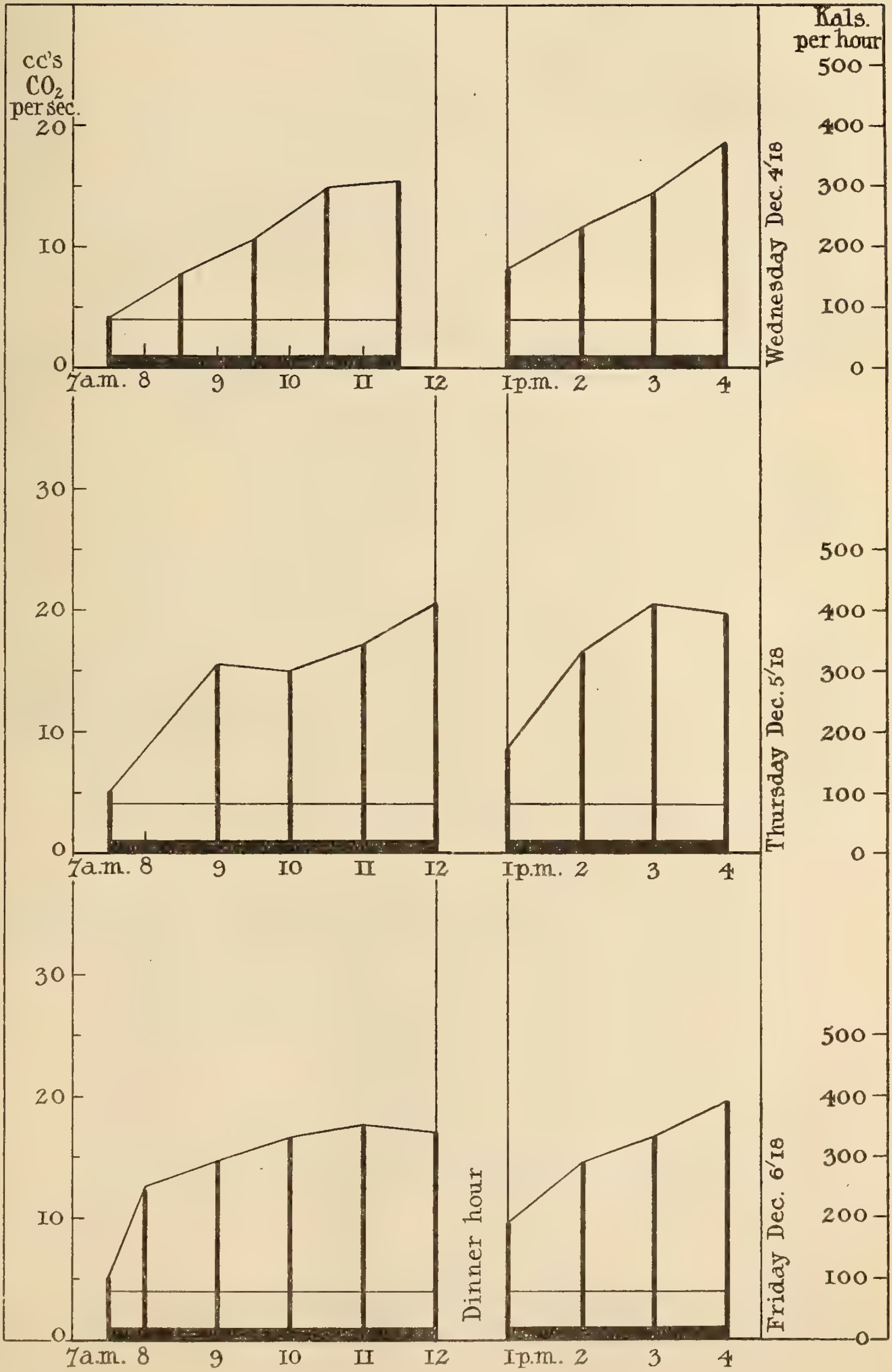
Forenoon, 17·02 Afternoon, 16·88.

General hourly mean for three days = 16·41
"Resting CO_2" = 4·00
Net cost of work = 12·41 c.c. CO_2 per sec.
= 248·2 Kals per hour.

Labourer No. 1.—Age 51. Weight (clothed), 84 kilos.; height, 1·71 metre (surface calculated = 1·96 sq. metre), East Surrey Docks. Three days' continuous observation.

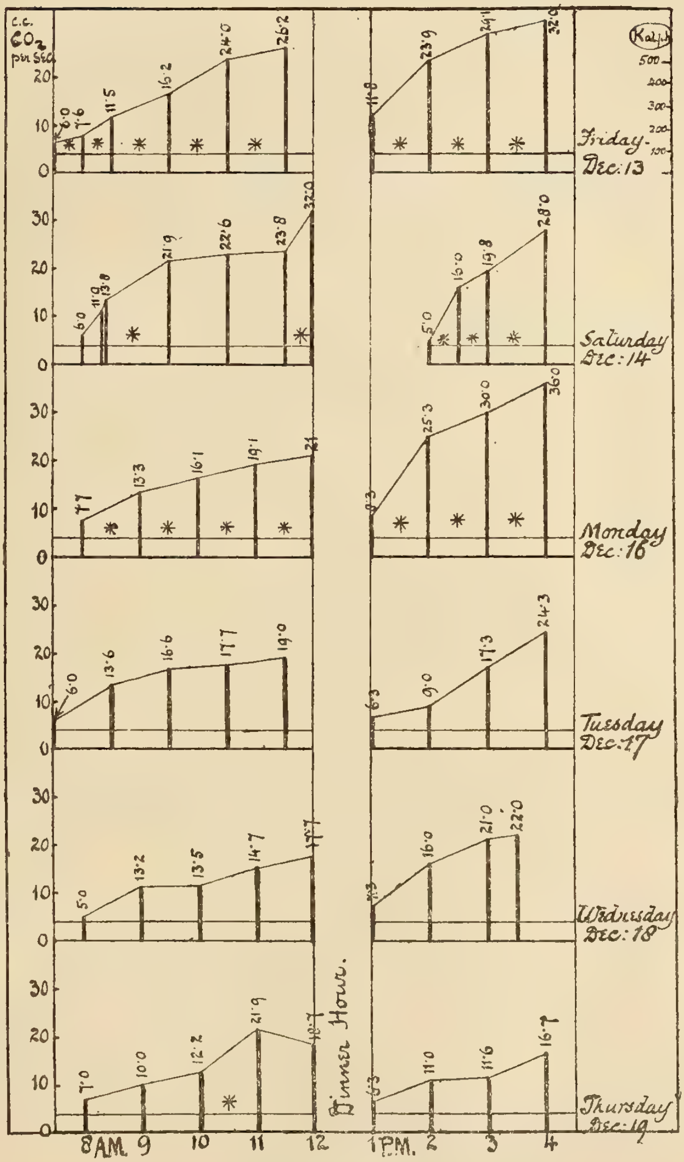
Time.	Time of sampling in seconds.	Ventilation in litres.	Ventilation in c.c. per sec.	CO_2 per cent.	CO_2 in c.c. per sec.	
7.30	60	11	183	2·4	4·39	Wednesday December 4, 1918. After ¼ mile walk followed by 5 mins. rest.
8.30	45	12	266	2·8	7·48	Laying a concrete floor. Chiefly "tapping" and trowelling.
8.33	60	13	216	2·3	4·96	
9.30	42	14	333	3·2	10·65	
9.33	60	12	200	2·8	5·60	
10.30	40	17	425	3·5	14·86	
10.34	60	14	233	3·0	7·00	
11.30	45	20	444	3·4	15·09	
11.34	60	11	183	2·8	5·12	
1.0	55	15	272	3·0	8 16	Immediately after ¼ mile fast walk.
2.0	43	18	418	2·8	11 70	"Tapping" and trowelling.
2.3	60	14	233	2·2	5·12	
3.0	45	20	444	3·2	14·20	
3.4	60	17	283	2·4	6·79	
4.0	43	20	448	3·8	18·54	
4.4	60	18	300	2·6	7·80	
7.30	60	12	200	2·5	5·00	Thursday, December 5. After ¼ mile walk and 5 mins. rest.
9.0	40	22	555	2·8	15·54	Finished with concrete floor at 10, when moved tools to another place, and prepared to lay another floor. Moving gravel and other building materials.
9.3	60	23	383	1·7	6·51	
10.0	42	21	500	3·0	15·00	
10.3	60	18	300	2·8	8·40	
11.0	40	22	550	3·1	17·05	
11.3	60	17	233	2·9	6·75	
11.45	40	23	575	3·6	20·70	
11.48	60	19	316	3·0	9·48	
1.0	60	18	300	2·8	8·40	After ¼ mile fast walk.
2.0	40	21·5	537	3·0	16·11	Laying concrete floor. Work rather heavier than usual.
2.3	60	20	333	2·6	8·65	
3.0	40	23	575	3·5	20·12	
3.5	60	19	316	3·2	10·11	
4.0	40	22	550	3·5	19·80	
4.3	60	20	333	3·0	10·00	
7.30	60	14	233	2·2	5·12	Friday, December 6. After ¼ mile walk and 5 mins. rest.
8.0	50	22	440	2·8	12·32	Laying concrete floor.
8.3	60	18	300	2·4	7·20	
9.0	40	21	525	2·8	14 70	
9.3	60	20	333	2·4	8·30	
10.0	40	22	555	3·0	16·65	
10.3	60	18	300	3·0	9·00	
11.0	40	24	600	2·9	17·40	
11.3	60	20	333	2·7	9·00	
12.0	40	23	575	3·0	17·00	
12.3	60	19	316	2·8	8·80	
1.0	60	21	—	2·8	9·80	Do.
2.0	45	22	—	3·0	14·64	
2.3	60	19	—	2·7	8·53	
3.0	40	20	—	3·3†	16·50	
3.3	60	16	—	3·1	8·24	
4.0	40	23	—	3·4	19·50	
4.3	60	20	—	3·0	10·00	

No. 1. Timework.



Labourer No. 2.—Age 58. Weight, 89 kgrm.; height, 1·75 M. Body Surface, 2·05 M². Six days' continuous observation.

Time.	Time of sampling (seconds).	Ventilation (litres).	Ventilation (c.c. per sec.).	CO_2 per cent.	CO_2 (c.c. per sec.).	——
Friday, December 13, 1918 (Piece-work).						
7.30	60	20	333	1·8	6·00	*Coaling, *i.e.*, heavy work against time.
8.0	50	19	380	2·0	7·60	
8.30	45	20	444	2·6	11·54	
9.30	40	23	575	3·0	16·25	
10.30	30	24	800	3·0	24·00	
11.30	35	23	657	4·0	26·20	
12 to 1 P.M. Dinner hour.						
1.0	60	22	367	3·2	11·74	*Coaling.
2.0	35	24	685	3·5	23·97	
3.0	30	23	766	3·8	29·10	
4.0	30	24	800	4·0	32·00	
Saturday, December 14.						
8.0	60	18	300	2·0	6·00	
8·30	30	15	500	2·2	11·00	Samples taken immediately before and after carrying a sack of coal.
8.32	30	16	533	2·6	13·80	
9.30	30	22	733	3·0	21·90	*Coaling; carried 21 sacks, 112 lbs. each, during the hour 8.30 to 9.30, distance about 10 yards downwards, 1/20.
10.30	30	20	667	3·4	22·70	
11.30	30	21	700	3·4	23·80	
12·0	30	24	800	4·0	32·00	*Coaling.
12 to 1 P.M. Dinner hour. Felt tired and took an extra hour off duty at midday.						
1.0	—	—	—	—	—	
2.0	60	15	250	2·0	5·00	
2.30	40	20	500	3·2	16·00	Heavy work. Preparing ground for building. Pounding. (Time scale.) Feels "very tired."
3.0	35	21	600	3·3	19·80	
4.0	30	24	800	3·5	28·00	
Monday, December 16 (Piece-work.) Still not feeling well.						
8.0	60	21	350	2·2	7·70	*Coaling.
9.0	30	20	667	2·0	13·34	
10.0	30	21	700	2·3	16·10	
11.0	30	23	767	2·5	19·15	
11.45	30	21	700	3·0	21·00	
1.0	60	20	333	2·5	8·32	*Coaling.
2.0	30	19	633	4·0	25·32	
3.0	30	21	700	4·4	30·00	
4.0	30	24	800	4·5	36·00	


c.c. CO2 per sec
Kalj.
500
400
300
200
100
0
10
20
30
Friday Dec: 13
6·0
7·6
11·5
16·2
24·0
26·2
11·8
23·9
24·1
32·0
Saturday Dec: 14
6·0
11·9
13·8
21·9
22·6
23·8
32·0
5·0
16·0
19·8
28·0
Monday Dec: 16
7·7
13·3
16·1
19·1
21
8·3
25·3
30·0
36·0
Tuesday Dec: 17
6·0
13·6
16·6
17·7
19·0
6·3
9·0
17·3
24·3
Wednesday Dec: 18
5·0
13·2
13·5
14·7
17·7
7·3
16·0
21·0
22·0
Thursday Dec: 19
7·0
10·0
12·2
21·9
18·7
6·3
11·0
11·6
16·7
Dinner Hour.
8 AM.
9
10
11
12
1 P.M.
2
3
4

Labourer No. 2—*continued.*

Time.	Time of sampling (seconds).	Ventilation (litres).	Ventilation (c.c. per sec.).	CO_2 per cent.	CO_2 (c.c. per sec.).	—
Tuesday, December 17 (Time work).						
7.30	60	18	300	2·0	6·0	Miscellaneous building work.
8.30	30	17	567	2·4	13·61	Shovelling, barrow-wheeling, brick-loading.
9.30	30	20	667	2·5	16·67	
10.30	30	19	633	2·8	17·72	
11.30	30	22	733	2·6	19·05	
1.0	60	20	333	2·2	6·32	Do.
2.0	30	18	667	3·0	9·00	
3.0	30	20	600	2·6	15·60	
4.0	30	23	767	3·2	24·54	
Wednesday, December 18 (Time-work).						
8.0	60	15	250	2·0	5·00	Building work. Concreting door-heads.
9.0	30	18	600	2·2	13·20	
10.0	30	15	500	2·7	13·50	
11.0	30	17	567	2·5	14·17	
11.45	30	19	633	2·8	17·72	
12 to 1 P.M. Dinner hour.						
1.0	60	20	333	2·2	7·32	Do.
2.0	30	15	500	3·2	16·00	
3.0	30	18	600	3·5	21·00	
3.45	30	18	600	3·8	22·80	
Thursday, December 19.						
8.0	60	21	350	2·0	7·00	Building work.
9.0	30	15	500	2·0	10·00	
10.0	30	16	533	2·3	12·26	
11.0	30	22	733	3·0	22·00	*Coaling from 11 to 12.
11.45	30	28	667	2·8	18·68	
12 to 1 P.M. Dinner hour. Says that the work has been easier and that he feels all right.						
1.0	60	18	300	2·1	6·30	Building work.
2.0	30	15	500	2·2	11·00	
3.0	30	14	467	2·5	11·67	
4.0	30	19	633	2·4	15·19	

The most remarkable "new fact" brought out in these observations is the progressive increase of the CO_2 ordinate in successive hours, which must be due to one or other of two alternatives, viz., increasing labour or decreasing efficiency.

In view of its gradual progression from hour to hour, it cannot be attributed to a discharge of accumulated CO_2, but is to be regarded as a true expression of the fact that the rate of internal production of CO_2 progressively increases. A progressively increasing discharge of CO_2 for such long periods, otherwise than in consequence of its increased production, is inconceivable, whereas it is quite reasonable to imagine that the animal machine at constant work loses efficiency with lapse of time, *i.e.*, does its work at an increasing cost, as expressed by its discharge of CO_2. Unless, of course, it should be due to increasing work.

Between the two possible causes, (1) increase of work, (2) decrease of efficiency by fatigue, I am inclined towards the second as the more probable. The root fact in fatigue is a consumption of material, presumably of carbohydrate in first instance, also of fat and of protein; subjective fatigue is felt when a certain degree of waste has been incurred. Objective fatigue commences long before its subjective aspect is experienced; it begins, indeed, with the beginning of work, with increased metabolism, of which increased discharge of CO_2 is the indicator and measure, and in the progress of the expenditure there is no line of demarcation to be drawn between a state of freshness and a state of fatigue; these terms are statements of the subjective states associated with the filled and unfilled reservoir of fuel. Moreover, the progressive increase takes place with piece-work under conditions where the work is of steady value.

For reasons that will be more fully developed, I hope, in a future report, I regard the slowly increasing ordinate during presumably constant work as a sign of the increasing cost of such work, *i.e.*, of the decreasing efficiency of the worker.

The hourly rise of CO_2 during a continuous spell of work has been in my experience a rule without exception under all conditions of work so far examined, and if peradventure a worker shows a fall in the ordinate one may be certain that a temporary remission of work has occurred just before observation.

In the present examples the hourly rise has been particularly well marked with piece-work, as shown in diagram, where the starred hours have been piece-work and the unstarred hours time-work. It is still more marked, though with less apparent regularity, in cold storage labour, which is less regular, but while it lasts, more exacting.

The cold storage workman, on duty from 1 A.M. to 7 P.M., is "standing by" through the night hours upon an hourly time wage that we may call basal.

As goods come in he is told off in gangs for piece-work, which brings him supplementary pay as his surplus. He wants to earn this surplus, and each workman does so under the automatic supervision of his mates in the gang, who are earning money in common; therefore he is working at full pressure, and the expired air collected from him at the end of each hour affords a fair sample of the level of CO_2 expenditure at which he is working. This expenditure is the important fact to ascertain; the result in cubic centimetres per second can afterwards be translated into calories per hour, and in this translation there is perhaps room for difference of opinion among different physiologists. I am accustomed for the mental arithmetic of the conversion to think of 1 c.c. per second CO_2 as representing 20 cals. per hour; but in case any other observer should be of a different opinion, and prefer to reckon from another respiratory quotient, I have given in the last column of Table VII Kalories per hour that correspond with different respiratory quotients. The further conversion of calories per hour per individual into calories per hour per square metre is, of course, readily accomplished by taking out from Tables (*e.g.*, that of Du Bois*) the surface values corresponding to the weight and height of the individual in question. Thus, *e.g.*, in the cases of Labourers No. 1 and No. 2:—

Labourer.	—	Net CO_2 per sec.	Net Kals per hour.	Surface in sq. metres.	Net Kals per hour per sq. metre.
No. 1	Time-work	12·4	248	1·95	127·2
No. 2	Time-work	12·3	246	2·00	123·0
	Piece-work	21·0	420		210·0

I do not like to estimate a day's work by taking the hourly estimate multiplied by hours of work. I think it preferable to plot the CO_2 ordinate of the whole day and to estimate the day's work from the area representing its net cost in cubic centimetres of CO_2 or in Kalories (*i.e.*, cubic centimetres of $CO_2 \times 20$). The hourly figures given above have been averaged from the three last hours of work, omitting the first reading as being possibly too low. Estimation of the area for the whole day includes, of course, the first reading and the hourly estimate obtained by dividing the day's value by the number of hours of work comes out lower than the hourly figures averaged from the last three hours. In the case of Labourer No. 2 the net cost of his six days' work calculated by area comes out as follows:—

---

* Du Bois and Du Bois, "Tenth Paper on Clinical Calorimetry," 'Archives of International Medicine,' vol. 15, Part II (May 15, 1915). (It is stated in this paper that Meet's formula $12{\cdot}313\ W^{2}/_{3}$ is more correctly stated as $10{\cdot}5\ W^{2}/_{3}$. This is the value I found by direct observation, 'Introduction to Human Physiology,' 1st edition.)

Labourer No. 2.	Net cost in Kalories.		Per hour, per sq. metre.
	Per day.	Per hour.	
Friday, December 13*	2431	324	167
Saturday „ 14†	1792	299	149·5
Monday „ 16*	2535	362	181
Tuesday „ 17‡	1568	209	104·5
Wednesday „ 18‡	1288	184	92
Thursday „ 19†	1155	165	82·5

* Piecework (coaling). † Mixed work. ‡ Time-work.

From these figures I read as the hourly cost of piece-work 175 Kals per hour per square metre; of time-work 100 Kals per hour per square metre, or 1400 and 800 Kals per day of eight hours.

The man-value of this labourer is considerably above that of the "average man" as defined by the Committee (weight 89 kgrm., surface 2 square metres). The allowance of 1900 calories made by the Committee for an average man should therefore, be increased by, say, 22 per cent. (27 per cent. according to weight, 17 per cent. according to surface), raising the allowance from 1900 to 2318. The total food-requirements of this labourer are thus:—

Per day (8 hours) of heavy work (time) $2318+1600 = 3918$
Of heaviest work (piece) $2318+2800 = 5118$,

or in round figures, 4000 and 5000 Kalories.

The mechanical value of the (eight-hour) day's work, costing 2800 and 1600 Kals, could be estimated if we knew the "physiological efficiency" of the worker. This was not measured for Labourer No. 2, but assuming that his efficiency was substantially the same as that measured for Labourer No. 1, viz., 25 per cent., the calculation is as follows:—

Net cost.	Work done.	Rate of work.
2800 Kals	700 Kals (= 297,500 kg.-ms.)	0·138 H.P.
1600 „	400 „ (= 170,000 „)	0·079 „

It will be instructive to compare these (high) figures with the lower figures arrived at by direct experiment on four O.T.C. cadets on staircase work (as given in a previous memorandum to the Committee on the Expression of Man-Power in terms of Horse-Power, September 30th, 1918). The pertinent figures given in this memorandum are as follows:—

1st Test: 25 Ascents of 20-metre Staircase in one hour.

Cadet.	Weight.	Total work done.	Kg. M. per sec.	H.P.
No. 1	63	31,500 kg. ms.	8·75	0·115
No. 2	57	28,500 ,,	7·92	0·109
No. 3	66	33,000 ,,	9·17	0·121
No. 4	56	28,000 ,,	7·77	0·102
		Average	8·20	0·112

2nd Test: 80 Ascents (1600 metres) in four hours.

Cadet.	Weight.	Total work done.	Kg. M. per sec.	H.P.
No. 1	—	101,520 kg. ms.	7·05	0·093
No. 2	—	92,720 ,,	6·43	0·084
No. 3	—	106,400 ,,	7·38	0·097
No. 4	—	88,060 ,,	6·11	0·083

All four cadets were obviously distressed during the last half hour, *i.e.*, the work was excessive.

3rd Test: 80 Ascents (1600 metres) in eight hours with ¼ hour rest at each hour and one hour for lunch at end of 40 ascents.

Cadet.	Weight.	Total work done.	Kg. M. per sec.	H.P.
No. 1	—	110,880 kg. ms.	3·85	0·051
No. 5	—	110,880 ,,	3·85	0·051

Neither cadet felt tired; both were quite willing to go on.

The conclusion drawn from these tests was that an average healthy young man, fit for military service, can do at least 100,000 kgrm.-metres per day, and that a mechanical task of 200,000 kgrm.-metres per day is beyond his power. I much regret that I did not then possess the simple apparatus necessary for keeping the CO_2 ordinate during these tests, and should be very glad if any "healthy young man" would volunteer to repeat the tests with control of his CO_2.

Conclusion.

The Food (War) Committee, in its 'Report on the Food Requirements of Man,' March, 1919, has proposed the adoption of the following classification of manual workers, in conformity with the excess of energy expended during eight hours' work over that expended during eight hours of sleep, as follows (p. 5):—

	Calories.
Sedentary	Less than 400
Light work	400 to 700
Moderate work	700 to 1100
Heavy work	1100 to 2000
(Requirements apart from work)	(1900)

These are "net" values, applicable to the daily sustenance of an "average man," defined in the Report as "an adult man of 66 kgrm. (unclothed), 171 centimetres in height,"* performing eight hours' average work in a climate such as that of France or England.

We have to examine how far the results observed on these two dock labourers, working under normal conditions, conform to the recommendation of the Food (War) Committee, and to collate them with the results of other observers associated in the inquiry. To make this comparison it will be necessary to reduce all results to a common denomination, preferably to the expression "calories per square metre per hour."

Averaged from the last three hours of forenoon and afternoon work (the first hour of work being omitted from calculation in order not to include readings taken before a steady *régime* has been established), the results in Kalories per square metre per hour come out as 127 and 123 for time-work, and 210 for piece-work. But estimates based on the last three hours to the exclusion of the first are probably too high, and it is preferable (*vide infra*) to estimate from the entire period of work inclusive of the first hour. The results on this basis are approximately 100 Kalories per hour for time-work and 160 for piece-work. With the exception of the last value, which represents a maximal value for the very heavy work accomplished in piece-work against time, the ordinary output for the heavy work done by these two labourers falls within the limits (78 to 142) contemplated for "heavy work" by the Food (War) Committee of the Royal Society,

These results should also be compared with those reported to the Committee by Rosenheim and by Greenwood.

I have found some difficulty in making this comparison in the case of Rosenheim's figures, which are for the most part given in terms of standard (basal) metabolism taken as 1.† To quote these figures as they stand would not afford any obvious comparison; I have therefore converted into Kalories per square metre per hour the averages of the principal data given on pp. 56 and 57 of his report, as follows:—

---

* For comparison with our observations which were made on clothed labourers, we have taken the average man as weighing 70 kgrm. clothed, with a body-surface of approximately 1·75 square metres.

† Rosenheim, "Prelim. Study of Energy Expenditure, etc., of Women Workers," 'Roy. Soc. Proc.,' B, vol. 91, p. 44 (1919).

—	Increase of standard.	Kalories per square metre per hour.
Light work	37·4 × 0·708	26·48
Medium work	37·4 × 1·090	40·74
Hard work	37·4 × 1·814	67·84
Walking (2·7 to 3·1 m.p.h.)	37·4 × 2·740	102·50

These are relatively low values of energy output, which is stated as having been much lower for "hard work" than for walking at about three miles per hour.

Greenwood and his collaborators conclude their report by the following estimate, but the conditions of observation described in the text do not appear to have afforded any opportunity of securing normal work data.* According to the weight of work, the subjects of observation are arranged in four groups:—

	Calories per sq. metre per hour.
Group I needing approximately	100
" II " "	125
" III " "	160
" IV " "	180

These appear to be gross values, inclusive of the values of basal metabolism. As stated in the report, the work was remarkably slack. Nevertheless from them the four corresponding work quotas for a seven-hour day: 1120, 1400, 1782, 2016 calories, which, augmented by 1410 for travelling and household work, with a 10 per cent. allowance for digestion, give as the final figures for the requirements of "strenuously employed industrial women":—

2810, 3120, 3555, 3805 calories.

These are surprising figures in view of the slackness of work that prevailed during Greenwood's observations. According to his conclusion, a "strenuously

---

* Greenwood, Hodson, and Tebb, "Report on the Metabolism of Female Munition Workers," 'Roy. Soc. Proc.,' B, vol. 91, p. 62 (1919). It is stated in the Report that the experiments were carried out during November, December, January, February, and March, 1918–19, in a factory, manufacturing 6-inch shell cases, and that in consequence of the relaxation of effort caused by the Armistice, the individual output was reduced by at least 50 per cent. . . . .

"The value of observations in this department (Tool Setting) is reduced by the notorious idleness of the operatives after November 11th. Often the work under experimental study was all the work done in the hour." (p. 67.)

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employed industrial woman," with a body surface of 1·6 square metres, requires nearly as much food-energy—3805 Kalories—as does our dock labourer during hard work, viz., 3918 Kalories.

The expression "work quota" might be understood to imply that the basal metabolism has been subtracted from the previous figures per metre, but, as stated on a previous page, the basal metabolism of the subjects was not measured. The gross values 100, 125, 160, 180 have, therefore, to be reduced by, say, 64 to give net results for comparison with our other data, viz., 34, 59, 96, 116.

With these modifications, which have been necessary for the purpose of reducing the figures to a common denomination, the various sets of values presented to the Committee may be briefly set out as follows:—

Net Kalories per square metre per hour.

Food (War) Committee of the Royal Society.	Rosenheim.	Greenwood, Hodson, and Tebb.	Waller and de Decker.
Sedentary ... under 26	— —	— — (gross)	
Light work ... 26 to 50	Light work ... 23·6	Group 1 34 (100)	— —
Moderate work 50 to 78	Moderate work 36·4	„ 2 59 (125)	— —
Heavy work 78 to 142	Hard work ... 60·4	„ 3 96 (160)	— —
		„ 4 116 (180)	Heavy work ... 100
			Do. piecework 175

In presenting this interim Report, I desire to thank the Port of London Authority for the liberal way in which it has placed its organisation at my disposal and enabled the work to be carried out with, I hope, the least possible interference with the workman's job. Adequate laboratory space has been placed at my disposal, and the courtesy of the officials and labourers with whom I have been brought in contact has made my task most agreeable.

I wish also to acknowledge the zeal, tact, and efficiency with which my assistant, Miss G. de Decker, has carried out the observations; they could not have been made at all without such assistance, which has assumed more and more the character of an independent enquiry, conducted and reported on by herself alone. This acknowledgment applies in fullest measure to the observations (Part II) of day-work and night-work carried out by Miss de Decker upon cold storage labourers.

The Genetics of "Rogues" among Culinary Peas (Pisum sativum).

By W. Bateson, M.A., F.R.S., and Caroline Pellew, Student of the John Innes Horticultural Institution.

(Received January 5, 1920.)

In 1915 we described observations and experiments on rogue peas. Our results up to the end of 1914 were as follows:—

(1) Rogues arise sporadically from self-fertilised seeds of various races of typical high-class peas.

(2) These rogues are characterised by pointed leaflets and upward-curving pods. Their foliar organs, stipules, leaflets, sepals, petals, and carpels are all narrower than those of the types. It is especially characteristic of rogues that their leaflets end in *points* instead of the emarginate apices proper to the types. Their seeds are, on an average, slightly smaller. Such plants have a curiously wild appearance, which contrasts greatly with the ampler proportions of the types.

(3) Rogues, self-fertilised, produce rogues exclusively.

(4) Rogues crossed with types, whichever way the cross be made, give F_1 plants which as seedlings show evident indications of the type-characters, having parts much larger than those of rogues at the same age. But these plants at an early stage, usually at some node below that at which the first flower is borne, *change to rogues,* producing stipules, leaves, and eventually pods, like those of rogues.

(5) The offspring produced by the self-fertilisation of these F_1 plants are *exclusively rogues.* In some way unexplained the type-characters contributed, whether by the father or by the mother, are excluded at an early stage, so that they do not reappear in the germ-lineage.

In the variety which we have chiefly studied, Sutton's Early Giant (a strain of the well-known Gradus), plants intermediate between types and rogues are not uncommon. We have scarcely ever seen such plants in other varieties. In them a change in the foliar parts occurs like that described in the F_1 plants; but though most frequently completed at or near the first flowering node, their transformation may be deferred to a much higher level. On such plants the shape of the pods also often changes progressively, lower pods being straighter and upper pods curved like those of rogues. These intermediate plants form a fairly definite class, though they grade insensibly into both the types and the rogues. In recording breeding results we have classified the plants into a graduated series of five groups, but for the present they may be reckoned as of two kinds: (1) those in which none of the leaflets have points; and (2) those in which pointed leaflets sooner or later appear. This in practice forms a convenient distinction which can almost always be applied. The two groups thus distinguished on somatic characters differ essentially in their genetic properties: "non-pointed" plants give on self-fertilisation what in previous publications we have called A families, consisting predominantly of types, or at least non-pointed plants, but the "pointed" give B families containing predominantly rogues. Families in which the proportion of the two kinds approaches equality occur but rarely.

Intermediate plants of composite or mosaic nature are not very uncommon, and the mosaicism may be of varying extent. In extreme cases, a whole branch may differ from the main stem, and a "pointed" branch giving a B family may arise from a "non-pointed" main stem giving an A family, or *vice versâ*. The adequate discussion of these irregularly composite plants must be deferred.

We have been chiefly occupied with a study of the genetic peculiarities connected with the gradual and regular assumption of the rogue characters by the intermediate plants which sooner or later acquire pointed leaflets. From the fact that the change was progressive, we surmised that if the position of origin of the seeds on such plants were determined, it would be found that the few non-pointed plants which they produce would be found to come predominantly from seeds in the lower pods, and the pointed from the upper pods. As the result of a preliminary trial we found that this rule was in fact followed, and this observation was published* at the end of 1915. Further experience has abundantly confirmed it.

But the converse conclusion which we also announced at the same time, to the effect that in the case of A families containing only a small number of rogues these come predominantly from the upper pods, has proved to be erroneous. In families where the pointed plants are proportionally few, the seeds from which they come are not derived from any particular level on the parent plants, and the observation on which we relied was only an accident of the special case.

Since the publication of these first results the work has been continued along several lines. Having established the fact stated above, that in the B families the non-pointed plants come chiefly from the bottom pods, we endeavoured to ascertain more exactly the positions from which they do come. Choosing, therefore, a number of intermediate plants which we thought likely to throw B families, we emasculated each flower on the stems in succession, as far as possible, pollinating them from type plants belonging to families which had been selected as being the most rogue-free.* Since, thus fertilised, every egg-cell genetically rogue, will produce a rogue, and every egg-cell genetically type will produce a type, the resulting family, sown in order, will show the distribution of the rogue- and type-characters among the egg-cells of the intermediate parent. The seeds from each pod were sown separately, and we expected to find that the non-pointed would come almost exclusively from the lowest pods. This expectation, however, was not borne out by the result, for the proportion of non-pointed among the egg-cells did not diminish noticeably at least up to the tenth flower. Since the same plants, if self-fertilised, very rarely produce a non-pointed plant from flowers higher than the fourth, a presumption was raised that the male and female sides must be differently constituted, and that the proportion of pointed gametes would be found to change with the level much more rapidly on the male side. Tests were therefore instituted, using pollen of the successive flowers of the intermediate plants and trying it on the ovules of types. The experiment has not been made on a scale sufficient to determine the true average ratios of non-pointed to pointed in successive flowers, but that the proportion of pointed increases rapidly from about the third flower is clearly proved.

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* 'Proc. Roy. Soc.,' B, vol. 89, p. 174.

Work of this kind is exceedingly laborious, and the wastage from various causes must inevitably be very large. Of the plants chosen for operation some necessarily prove to be of the wrong kinds, throwing, for example, all rogues and all the work done on such plants turns out in the following year to be inapplicable. We considered ourselves fairly fortunate in having raised 1016 cross-bred plants from which to estimate the output of the female side, and 468 plants derived from the male side of the pointed intermediates, together with 1572 plants from flowers of known position self-fertilised.† The results are set out in Table I, where the actual numbers from each flower are given with the ratios deduced from them. As to the female side of the flowers the evidence may be regarded as significant up to the 14th flower. The proportion of non-pointed female gametes remains about constant at a ratio of rather more than 1 in 2 up to the tenth flower, above which it diminishes. On the male side the proportion of non-pointed gametes is about 1 in 5 for the first two flowers and then drops sharply and progressively in succeeding flowers, falling again suddenly after the fifth flower. We are satisfied that the record for the eighth flower is quite abnormal, and may be disregarded. Further observations will no doubt modify the figures here given, but the general trend of the results is unmistakable.

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* The proportion of rogues (or, more strictly, pointed) thrown by ordinary types is, in our experience, about 2 per cent. In the purer type-families selected for crossing the percentage would be even lower than this, so that for practical purposes they may be regarded as rogue-free.

† An attempt was made on a considerable scale, by sowing the peas of each pod in their original order, to test whether there is any orderly arrangement of the seeds. This work, which added greatly to our task, led to no positive result.

Table I.—Offspring of Pointed Intermediate Plants (selfed or crossed with type) grouped according to the ordinal position of the flowers from which the seeds were taken.*

Ordinal No. of flower.	Cross-fertilised.						Self-fertilised.		
	Type-father.			Type-mother.					
	Non-pointed.	Pointed.	Ratio.	Non-pointed.	Pointed.	Ratio.	Non-pointed.	Pointed.	Ratio.
1	57	54	1 : 1·09	9	42	1 : 4·6	18	269	1 : 15·0
2	30	42	1·4	17	83	4·9	21	240	11·4
3	65	46	0·7	8	56	7·0	18	231	12·8
4	56	64	1·1	3	30	10·0	15	228	15·2
5	32	46	1·4	7	86	12·3	4	218	54·5
6	26	37	1·4	2	59	29·5	—	144	
7	48	43	0·9	1	23	23·0	1	91	
8	34	41	1·2	6	28	4·6	—	71	
9	34	46	1·3	—	8				
10	25	27	1·1						
11	10	31	3·1						
12	15	23	1·5						
13	10	23	2·3						
14	5	27	5·4						
15	2	8							
16	3	3							
17	2	1							
	454	562	1 : 1·2	53	415	1 : 7·8	77	1492	1 : 19·4

The series on the female side is much longer, because the fertilisations were largely done in 1916, a year in which the plants grew very tall. The height of a plant depends greatly on the weather, and in 1917–19 short plants were the rule. Naturally it can only be on tall plants that the contrast between the composition of the offspring derived from the lower and the upper pods can be fully displayed. In Table II a number of such families are set out. To save space and labelling the pods were generally

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* The material used in this Table is necessarily somewhat heterogeneous. It includes offspring of plants which, whether selfed or crossed, have given at least one plant as high as Class 3. To have extended it lower would have involved the inclusion of families from plants giving all rogues, and such plants could not exhibit the gradational effect which would thus have been to some extent masked. Nevertheless, a few of these ought, no doubt, to have been included as genetically comparable. The figures given for the results of self-fertilisation are not all derived from the plants actually used for the crosses, but include any results from flowers of known position borne by comparable plants.

Table II.—Families produced by Pointed Intermediate Plants (self-fertilized) which gave a small minority of non-pointed members. Results tabulated according to the flowering nodes from which the seeds were taken.

Register No. of parent.	Ordinal No. of flowers.	Classes.				
		Non-pointed.		Pointed.		
		1.	2.	3.	4.	5.
$36^{1}/6$ Main stem	1–7	—	1	—	—	28
	8–11	—	—	—	—	27
	12–17	—	—	—	—	28
Branch from 7th node	1–5	—	6	1	2	22
	6–8	—	1	—	—	20
	9–15	—	—	—	—	39
$41^{3}/6$ Main stem	1–4	—	5	5	—	9
	5–8	—	1	—	—	13
	9–12	—	—	—	—	16
	13–15	—	—	—	—	16
Branch from 9th node	1–3	—	—	6	—	8
	4–6	—	—	—	—	17
	7–9	—	—	—	—	22
$178^{4}/6$	1–4	—	1	—	3	12
	5–9	—	1	3	—	21
	10–14	—	—	—	—	25
$178^{7}/6$ Main stem	1–4	—	4	—	—	16
	5–8	—	—	—	—	22
	9–15	—	—	—	—	26
Branch from 8th node	1–7	—	4	2	—	33
	8–11	—	—	—	—	15
$192^{1}/6$	1–6	—	4	2	—	11
	7–9	—	—	—	—	22
	10–14	—	—	—	—	21
	15–19	—	—	—	—	24
$205^{3}/6$	1–5	1	1	—	3	18
	6–9	1	2	2	—	23
	10–15	—	—	2	—	35
$258^{1}/6$ Main stem	1–8	—	1	3	—	31
	9–13	—	—	—	—	28
	14–21	—	—	—	1	35
Branch from 8th node	1–2	1	3	—	—	8
	3–7	—	—	1	1	36
$349^{2}/6$	1–7	3	4	7	—	11
	8–13	—	—	—	—	33
	14–15	—	—	—	—	10
$278^{1}/6$	1–5	—	7	8	—	11
	6–9	—	—	6	—	25
	10–16	—	—	1	2	11

Table II—*continued.*

Register No. of parent.	Ordinal No. of flowers.	Classes.				
		Non-pointed.		Pointed.		
		1.	2.	3.	4.	5.
$25^6/7$	1–2	2	—	2	—	6
	3–5	—	—	—	—	15
	6–7	—	—	—	—	17
$112^1/17$	1–2	3	3	1	1	1
	3–4	—	3	—	4	1
	5–6	—	—	—	—	9
	7–8	—	—	—	—	3
$183^1/7$	1–2	—	1	—	—	14
	3–4	—	—	—	1	12
	5–7	—	—	—	—	16
$185^5/7$	1–2	2	4	2	—	1
	3–6	1	3	10	5	9
	7–8	—	1	3	3	3
	9	—	—	—	1	3
$265^1/7$	1–3	—	5	—	2	7
	4–5	—	—	—	1	7
	6–7	—	—	—	—	8
$453^1/7$	1–2	—	2	3	1	3
	3–4	—	—	1	1	7
	5–7	—	—	—	—	9
$457^1/7$	1–2	—	1	—	—	9
	3–4	—	—	—	—	11
	5–6	—	—	—	—	5
	7–8	—	—	—	—	12
	9–11	—	—	—	—	5
$254^1/8$	1	—	—	—	1	6
	2–3	—	1	1	1	8
	4	—	—	—	1	8
	5–6	—	—	—	1	15
	7–10	—	—	—	1	22
$373^4/8$	1	—	1	1	1	1
	3–4	—	—	—	—	9
	5–6	—	—	—	—	14
	7–8	—	—	—	—	11
$556^1/8$	1–3	1	4	1	2	12
	4–5	—	1	1	4	7
	6–8	—	—	2	2	10
$603^1/8$	1	—	—	—	—	7
	2–4	—	1	—	—	13
	5–8	—	—	—	—	19
$621^9/8$	1–2	—	1	—	—	13
	3–4	—	—	—	—	13
	5–9	—	—	—	1	22

grouped into three or four successive lots. This was done before we set about determining the actual ratios for each flower, and though the numbers are not available for that purpose, they provide a larger total of observations than could have been reached had the material been more subdivided. It will be noticed that a progressive decline (towards the rogues) can be often seen in the offspring of *branches* as well as on the main stems.

In Table II the plants are classified in the five classes which we have arbitrarily used. Class 1 is the true type; Class 2 differs from it slightly and evasively in foliage, but chiefly in having some curvature in the pods; Class 3 are the pointed intermediates of the kind which show the gradational changes; Class 4 contains plants below Class 3 and approaching Class 5, which last are the genuine rogues. Of these classes, 1 and 2 are non-pointed; 3, 4, and 5 are pointed. There is a complete series of intergrades, both in somatic appearance, and in genetic constitution as indicated by the families produced.

For Table II we have chosen those families which contained at least one non-pointed plant. The progressive decline towards rogues affects the lower classes, and not merely the frequency of the non-pointed plants. The same feature is also shown by the families containing only pointed plants, which we have not included in this epitome. It appears also in the distribution of the several pointed classes resulting from crossing the intermediates as female with type, the pointed plants thus produced by the *lower* pods being chiefly Classes 3 and 4, the corresponding plants from the upper pods being chiefly rogues of Class 5. This feature, which is not shown in Table I, was most conspicuous when the tall intermediates were used.

Inasmuch as the relative proportions of the several kinds of offspring produced by Class 3 plants (and doubtless by Class 4 also) depend on the absolute height of the parents, it is impossible to give any significant figure for these proportions, but the averages produced by the self-fertilisation of the several classes are roughly as follows:—

	Non-pointed	to	Pointed.
Class 1	47	......	1
„ 2*	12	......	1
„ 3	1	......	10–20
„ 4	1	......	160
„ 5	0	......	All rogues.

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* Class 2 in our first paper ('Jour. Genetics,' vol. 5) was not the exact equivalent of that subsequently adopted. We had not then appreciated that the points are the best criterion, and we previously included in Class 3 plants which we now know to belong genetically rather with Class 2. The inclusion of such plants in Class 2, of course, lowers the average offspring of that class.

Since plants can be found giving every proportion of non-pointed to pointed, the actual ratios observed in any group of plants mean little unless the group can be accurately defined. Such definition on somatic characters is not strictly possible. In choosing plants for crossing, we took those which, judged on their *early stages*, might be expected to throw a small minority of non-pointed plants, for our object was to examine the gradational effect, which can only be manifested by plants of that composition. In Table II, however, we have plants chosen when ripening, on their *adult* characters, selected further on account of their being well grown, and the result is that these, as tested by their offspring, proved to be of a somewhat higher constitution than those chosen for crossing. For any plants which did eventually show the rogue "points" are not too high to show the gradational effect, though judged as young plants, before they had assumed the points, they would not have been known to be available for the purposes for which the crosses were made. The higher the level at which the points appear, on the whole, the higher is the proportion of non-pointed plants found among the offspring, but this correspondence is rough, and only becomes apparent when long series of families are examined.

Recapitulation.

The three chief phenomena may be recapitulated:—

1. Reciprocal crosses between type and rogue give plants which, as they develop, turn into rogues.

2. Though the characters of the type are certainly introduced, manifesting their presence by affecting the form of the young F_1 plant, they very rarely* take part in the germ-lineage, being apparently left behind in the lower nodes.

3. Plants really intermediate between type and rogue nevertheless exist, but never breed even approximately true. Their germ-cells may be either type, intermediate (of at least two kinds), or rogue. The proportion of gametes carrying type-characters is different on the male and female sides. In both sexes the ratio shows gradational change.

Of the egg-cells of the lower flowers, up to about the 10th flowering node, rather more than 50 per cent. carry the type-characters—or, at least, the non-pointed character—above which level the proportion declines.

Of the pollen in the lowest two flowers, only about 20 per cent. is type-bearing, and the proportion diminishes rapidly in each successive flower above this level.

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* The exceptions mentioned in our first paper ('Jour. Genetics,' vol. 5, pp. 29–30, 1915), should probably be thus interpreted. A few others have since been met with which hereafter will need detailed description.

Discussion.

Features somewhat comparable with the first two of these peculiarities have been recorded, but the third is, so far as we are aware, as yet without parallel. Biffen* has observed the total "suppression" of a character, grey chaff, introduced by Rivet wheat, in a cross with Polish; and, in variegated *Capsicum*, crossed with green, Ikeno† never recovered the green in later generations. Baur‡ interpreted certain wholly white and wholly green offspring obtained in crosses between white-skinned and green plants by the very probable suggestion that the green or white characters might have been omitted by somatic segregation, though the inference was scarcely capable of direct proof. Winge§ also saw something possibly analogous in *Humulus*, traces of variegation appearing in lower leaves of plants, which subsequently became green. None of these examples, however, are strictly comparable, but Winge's case perhaps comes nearest.

A *gradational* change in the numerical proportions of the gametes at the successive nodes has not, we believe, been elsewhere observed. The distribution of the type gametes borne by the pointed intermediates corresponds, no doubt, in a loose way with the distribution of vegetative vigour. But weak growth on type plants is not specially likely to bear rogues, nor do rogues, however luxuriantly growing, produce types. Aetiological interpretations of this kind are inconsistent with all that we have learnt of genetic principle. Nevertheless, the coincidence may not be without significance.

Where the output of the several sorts of gametes is so uncertain no gametic system of a Mendelian kind can be propounded. The most that can be expected of such a system in this case is that it should qualitatively represent the distinction between the genetic nature of the classes here called 2 and 3. Since type (T) and rogue (R) gives F_1 rogue, neither of these intermediates can receive a rogue gamete in fertilization. Since also these two classes differ from the type, the gametes composing either of them cannot *both* be type-bearing. From this reasoning it appears to be practically certain that two sorts of intermediate gametes must exist, the one more type-like, T′, the other more rogue-like, R′. TT′ will then represent Class 2 and TR′ Class 3; but where, as in this case, there is no clear discontinuity no analysis can be pressed. All that can be positively stated is that there are two sorts of intermediate gametes, that both are unstable, being incapable of constituting a stable zygote, and that when united in fertilization with a type gamete, the resulting zygote is nearer the type than any combination of intermediate gametes would be.

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* 'Jour. Genetics,' vol. 5, p. 227 (1916).

† *Ibid.*, vol. 6, p. 201 (1917).

‡ Especially 'Zeits. f. ind. Abstammungslehre,' vol. 4, Heft 2 (1910).

§ 'C. R. Lab. Carlsberg,' vol. 14, No. 3, p. 11 (1919).

Class 2 plants throw perceptibly more pointed plants than do the real types (see p. 192) but they do not show the gradational phenomenon as Class 3 plants do. Evidently the gradual extrusion of the type at the successive nodes must be a process similar to that by which the type is extruded in the base of F_1, differing only in that it is prolonged over a longer series of nodes. The actual data suggest that the gradational phenomenon occurs with greatest intensity in the more rogue-like of the intermediate plants and is less well marked in those which, judged by their offspring, are higher (more type-like) in genetic composition, being absent altogether in Class 2. Discussion of these points cannot be given in brief.

As it seemed possible that the types might be tetraploid, counts of chromosomes were made by Miss Nesta Thomas, but the number found in both rogue and type was the same (seven for the haploid number).*

The persistent recurrence of rogues among the offspring of types must indicate some liability to an error in cell-division. Once the abnormality has occurred, of which pointed leaflets are the ostensible indication, there is a progressive change in successive generations such that, assuming equal fecundity in all classes, the progeny would in a few generations consist of rogues in overwhelming proportions. It was a commonplace of practical breeders and of conventional evolutionists that when selection is suspended, a breed "degenerates." This doctrine, promulgated, as it commonly was, without any reservation as to crossing or reference to critical purity of line is fallacious as an expression of physiological truths however much the objective consequences may seem to fulfil the prophecy. In the present example the popular conception of degeneration is precisely realised. So far as we know it unique.

* Since confirmed by Prof. K. Matsui.

The Properties of Colloidal Systems. IV.—Reversible Gelation in Living Protoplasm.

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Protoplasm itself, using the name to express living substance in its simplest, undifferentiated form, is generally recognised as having the properties of a somewhat viscous liquid. This fact was realised as long ago as 1864 by Kühne (1864). As usually seen under the microscope, it contains suspended in it a number of granules of a great variety of dimensions and properties. But in the pseudopodia of an amœba or of a leucocyte, when examined by the ordinary method of illumination with transmitted light, it appears completely devoid of contents or structure. As Hardy (1899) showed, the various networks and similar arrangements seen in fixed preparations are produced by the action of the reagents used, although they indicate that protoplasm contains matter in the colloidal state.

The use of the method of brilliant lateral illumination on a dark ground (so-called "ultra-microscope") has led to the detection of particles in protoplasm which are too minute to be visible by ordinary illumination. Of course, these particles, being comparable in dimensions with the mean wave-length of light, are not seen in their true dimensions or form, but by their diffraction discs. Remembering that the late Lord Rayleigh showed that the more intense the illumination, the more minute are the particles that it is possible to detect, I tested the result of increasing the intensity of the dark-ground illumination applied to the apparently clear and structureless pseudopodia of large amœbæ. The broad flat pseudopodia of a species, which appeared to correspond to *Amœba princeps* (Leidy), were found to be most appropriate for the purpose. A paraboloid condenser, made by Zeiss, was used in most cases. The source of light was the positive crater of a small arc lamp with carbons at right angles to one another. The rays were made parallel by a condenser, and passed through a cell with parallel sides, about 5 cm. apart, before falling on the mirror of the microscope. The water-cell was found to be necessary on account of the heat otherwise transmitted being sufficient to kill the organisms. In order to obviate the injurious effect of any ultra-violet rays which might be transmitted through the system, quinine sulphate was added to the water. The objective used for the majority of the observations was an excellent $\frac{1}{6}$-inch dry lens made by Swift, which was found to admit of magnification by fairly high-power oculars, such as No. 12 compensating of Zeiss. Other methods, such as that of an objective as sub-stage condenser, with a central stop in the observing objective to cut out direct rays, were tried; but the paraboloid was found to be the best on the whole.

In practice, the observations themselves are somewhat trying, on account of the extreme brilliancy of the larger particles when they come into the field of view. But under favourable conditions, and with sufficient intensity of illumination, it is not difficult to see that the clear protoplasm of the pseudopodia contains an immense number of very minute particles, shown by their bright diffraction images. They are in vigorous Brownian movement, and it is scarcely possible to distinguish separate particles, on account of their number. The general appearance is that of a shimmering tremulous movement in the field of view.

This Brownian movement is one of the most convincing proofs of the liquid nature of the system. A simple experiment shows the fact. If a cake of water-colour gamboge be rubbed in a drop of 5 per cent. gelatine solution on a warmed microscope slide, covered and immediately examined under the microscope, the particles exhibit Brownian movement in the usual manner. As the slide cools and the solution sets into a jelly, the movement becomes inert and finally ceases, the particles becoming fixed in the meshes of the network. On warming, the movement reappears. The fact was made use of by J. Duclaux (1908) for the purpose of enumerating the particles in colloidal ferric hydroxide.

It seems, from Kühne's description of his valuable observations, that he was unable to satisfy himself that the particles which were visible to him possessed Brownian movement, or, as he called it, "molecular movement," as distinct from the translational movements due to currents in the protoplasm. If he had been able to use dark-ground illumination, there is no doubt that he would have detected them, since they can be distinguished by the vibratory motion of the particles even when they are being carried along. They have, indeed, been described by Gaidukov (1910) and by Price (1914) in plant cells, by Chambers (1917) in various animal cells, by Mott (1912) and by Marinesco (1912) in nerve cells.

Protoplasm belongs, then, to the class of colloidal solutions called by Graham (1864) "hydrosols." Now, as Graham showed, many of these under certain conditions change their state, becoming solid, in the sense that they become fixed in shape. This phenomenon is familiar in the "setting" of gelatin when cooled, and the new systems are called "hydrogels." The series of changes taking place was investigated by Hardy (1900). It is sufficient for our present purpose to call attention to the fact shown by the experiment with gamboge given above, namely, that liquid properties cease and a fixed structure makes its appearance.

Certain facts noticed by Kühne, in his experiments on electrical stimulation of protoplasm, suggest that a reversible change of the kind referred to may occur in this colloidal system, although Kühne did not interpret them as of such a nature. It is to be remembered that his work was done very soon after the publication in 1861 of Graham's investigations on colloids which had not then become a part of common physiological knowledge. An observation made by Gaidukov (1910, p. 58), in the course of his work on the phenomena shown in living protoplasm by dark-ground illumination, suggests that "spontaneous" changes from sol to gel take place. This observer noted that the movements of the protoplasm in the cell of *Vallisneria* appeared occasionally to become arrested at a spot, while the Brownian movement of the particles in it ceased. Presently, the Brownian movement became visible again, and immediately afterwards the general protoplasmic circulation was resumed. According to Chambers (1917) there is a periodic reversal of the sol to the gel state, and *vice versâ*, in the process of cell-division, the greater part of the cell being in the state of gel when the aster is fully formed. In fact, the appearance of the aster is associated with gelation of the protoplasm. Leblond (1919) describes the phenomenon as occurring in conjugation, as well as in cell-division. In fact, the occurrence of reversible gelation seems to be a regular condition of cell activities. Some observers have described the appearance of a reticular structure. But, on account of the ease with which a series of points gives rise by diffraction to network images, care is needed in the interpretation of such images. Nevertheless, Hardy (1900) has described the formation of networks in moderately strong solutions of gelatin in dilute alcohol, so that there is reason to admit the possibility of their production in protoplasm.

The experiments which I made were for the purpose of seeing whether this reversible state of gelation could be induced at will by means of electrical stimulation. The method used for stimulation was the same as that of Kühne. Two thin blunt-pointed pieces of platinum foil were cemented on a microscope slide, so that there was a gap of about 2 mm. between the points. A drop of water containing amœbæ or other cells was placed between the points and a cover-glass dropped on the top. The platinum strips can readily be connected to the terminals of the secondary coil of an induction apparatus by resting the bared ends of fine copper wire on them and placing small weights on these. It was found to be a matter of some difficulty to adjust the strength of the stimulus to an appropriate value. If too strong, the organism "explodes," driving out its contents into the water, where they rapidly become dispersed. But even if the current is not strong enough to produce any immediate effect, it is necessary to cut it off the moment that any trace of contraction or other effect shows itself. A very weak current, if left on for a sufficiently long time, kills with disintegration. Moreover, the protoplasm may be "killed," in the sense of permanent cessation of movement, although no immediate breaking up may take place. Since, therefore, if recovery is to be expected, it is not permissible to continue the stimulus for more than a brief time, the observations must be made rapidly.

It is a comparatively simple matter to convince oneself of the correctness of Kühne's statement with regard to the cessation of the flowing movements of the larger granules into and out of the pseudopodia. This is, no doubt, rightly stated to be due to the "contraction" of the organism into a more or less spherical shape, with simultaneous arrest of the protrusion and retraction of the pseudopodia. But it is clear that this observation does not necessarily imply a change of the protoplasm into the gel state. This latter can only be tested by examination of the Brownian movements of the minute particles visible by dark-ground illumination. The most satisfactory place to make this observation is the clear protoplasm of the outer part of the pseudopodia, which is free from the to-and-fro movement of the large granules of the central protoplasmic mass. It is generally possible to make use of a stimulus not strong enough to cause sudden retraction of the pseudopodium as a whole. In a successful experiment, the effect is very striking. The continuous shimmering tremulous movement of the bright points, due to their Brownian movement, ceases almost instantaneously, as if the liquid protoplasm had been frozen. As soon as this happens, the stimulation is stopped, and, apparently, almost at the same time, the Brownian movement and the flowing pseudopodial extrusion recommence.

It is significant that the Brownian movement does not cease during natural pseudopodial movement of the protoplasm. This fact might, perhaps, be regarded, especially by those who look upon the gel state as an accompaniment of cell activity in general, as evidence that the protrusion of pseudopodia is the result of differences of surface tension at the contact between protoplasm, water, and solid surface on which the organism rests. But the mode of production of pseudopodia is as yet a matter of dispute.

If the electrical shock in the experiments described above has been too strong, so that the organism is killed, but not so strong that "explosion" takes place, the fixed state of gelation is permanent; the sol state does not return until disintegration sets in. This is in agreement with the statement of Gaidukov that death is associated with an irreversible coagulation. It appears, however, from some observations of Kühne, that the lethal gel state begins at a later stage to show Brownian movement again. Kühne interpreted this reappearance as being due to absorption of water. It may indicate the commencement of autolysis. Sherrington (1894, p. 188), in his observations on leucocytes, regarded the appearance of Brownian movement as a sign of approaching death. It seems probable that the second stage may come on more quickly in these cells than in *Amœba.* Leucocytes may consist of more viscous protoplasm and the granules observed by Sherrington may have been too large to show Brownian movement until the post-lethal changes had reduced the viscosity of the medium. I have not made any observations on these cells. Pus cells, according to Sherrington, show Brownian movement of the particles which they contain.

Although many plant cells are very favourable objects for the investigation of the movements of protoplasm, I found them less so for the brilliant illumination required for the observations of the present paper. The cell wall, by its dazzling brightness, renders the detection of changes in the protoplasm in contact with it a matter of difficulty. In *Nitella,* probably owing to the relative delicacy of the cell wall, I have, however, been able to observe phenomena similar to those in *Amœba.* In *Spirogyra,* the central vacuole contains numerous granules in Brownian movement, and I was unable to satisfy myself that their movement could be distinguished from that of the particles in the protoplasmic layer itself. In the staminal hairs of *Tradescantia,* although one could recognise a cessation of Brownian movement in the protoplasmic trabeculæ on stimulation, it would have scarcely been possible to feel assured of it without previous acquaintance with its appearance in *Amœba,* for the reason referred to in the case of *Spirogyra,* namely, the presence of particles in the cell sap, on which stimulation has no effect.

Summary.

With intense dark-ground illumination it is possible to see that the apparently clear pseudopodia of *Amœba* are filled with numerous very minute particles in Brownian movement; thus affording further evidence of the liquid, hydrosol, nature of simple protoplasm.

By electrical stimulation, this sol can be reversibly changed into the gel state, evidenced by the sudden cessation of the Brownian movement.

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Studies of Photo-synthesis in Fresh-water Algæ.—1. The Fixation of both Carbon and Nitrogen from the Atmosphere to form Organic Tissue by the Green Plant Cell. 2. Nutrition and Growth produced by High Gaseous Dilutions of Simple Organic Compounds, such as Formaldehyde and Methylic Alcohol. 3. Nutrition and Growth by means of High Dilutions of Carbon Dioxide and Oxides of Nitrogen without Access to Atmosphere.

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The two most primæval and most fundamental chemical processes for living organisms are those two in which their living substance is synthesised from inorganic sources with uptake of energy. By one of these carbon is built into organic compounds, starting with the oxidised carbon dioxide of the atmosphere and utilising the energy of sunlight. The source of the inorganic nitrogen, which in the second is likewise built into organic forms in the amino-acids and proteins, is more obscure, and has in the course of 150 years led to much disputation. In the present and succeeding papers evidence will be adduced that the source of the nitrogen utilised by the plant does not lie in the soil (although a luxury or *luxus* supply may be given from the soil), but in the air, and that the reaction by which the atmospheric nitrogen and oxygen are made reactive is a photo-synthetic one, in which the energy of sunlight is absorbed and converted into chemical form as nitrites in the green cell.

This view places these two processes of carbon and nitrogen assimilation upon the same basis, and make them coeval in the process of evolution; this, as will presently be pointed out, must have been the case, in order that any living organism could ever have appeared upon the earth.

The importance of this question of the fixation of nitrogen has induced much study of it and led to many polemics, and it is interesting how invariably the weight of scientific authority has been upon the wrong side. Eminent men, supporting error unconsciously with negative experiments, have stated that the nitrogen was obtained from the soil and never by the leaves from the air; less-known men, backing their statements by positive proofs, were on the other side, but the eminence and authority of their rivals was too much for them, and so it comes about that in this matter we still almost universally believe and teach " the thing that is not."

It was Priestley himself, the discoverer of the like process for carbon assimilation, who in 1771 first asserted that plants were able to absorb the nitrogen of the air, and Ingenhousz shortly after endorsed this statement. But this earlier view was contradicted, first by de Saussure, and later by Woodhouse, Senebier, and then by the eminent agricultural chemist, Boussingault, in France, and the no less eminent Lawes, in England.

Ville, in France, stoutly maintained that plants absorb nitrogen from the air, and his findings were confirmed by a specially appointed Committee of the Académie des Sciences. Boussingault repeated his own experiments with the utmost care and every precaution, and again obtained negative results. This was later confirmed in an elaborate series of researches made by Lawes at Rothamsted; and the eminence and great authority of these two distinguished men settled the controversy in their favour for a generation.

Experiments that we have ourselves made in the course of the present investigation, as to the growth of the seeds of higher plants (mono- and dicotyledons) in sand and water cultures, upon media free from compounds of nitrogen, have convinced us that the experimental results of these observers were correct, but their deductions profoundly erroneous. The seedlings we obtained were dwarfs, although they flowered and seeded, and they assimilated scarcely any nitrogen. This does not by any means settle the problem; it only proves that, so far as the higher plants are concerned, this is an abortive method of experimentation. If the conditions of enviroment are such as to make any feeble growth pathological, and if the dry weight of the plant scarcely exceeds that of the seed from which it grew, it is scarcely to be expected that there should be an increase in nitrogen. On the other hand, had the seeds grown into anything like normal plants, it would have been an unthinkable monstrosity in nature that carbon, hydrogen, and oxygen should go up without any accompanying increase in the nitrogen.

It has been shown by Jamieson (1), in several species of higher plants, that when a minimal amount of nitrogen is supplied, these grow, increase in weight, and form far more combined nitrogen both in soil and plant than has been supplied in original soil and added media. These experiments of Jamieson's were carried out with the utmost care, attention being paid to every possible source of gain or loss of nitrogen. Moreover, they are supported by several other series of observations, such as gain of nitrogen in aquatic plants with no soil roots, growth of large trees rooted in clefts in basaltic and granitic rocks, where the total nitrogen in the soil could not afford sufficient for one annual crop of leaves, analysis for nitrogen of plants found growing in hard mortar in walls where the amount of nitrogen available, except from the atmosphere, was practically negligible.

A careful perusal of the wealth of facts published by Jamieson in the 'Annual Reports of the Aberdeen Agricultural Research Association,' against which there are only to be set these negative results of Boussingault and Lawes, is convincing to us as to the positive solution of the problem in favour of the assimilation of nitrogen from the air by the green cell. These results have been confirmed by Mameli and Pollacci (2) at Pavia; they are reinforced by the experiments in this paper upon Fresh-water Algæ, and also by others shortly to appear made upon Marine Algæ.

Our own approach to this problem has been from a different point of view from those who have preceded us; they have been engaged in its consideration in relation to the bio-chemistry of the plant, or its importance in nutrition in agricultural chemistry; we have been investigating the matter from the point of view of the primæval origin of living organisms, before there was yet anything so complicated as a green cell upon the earth, and when organic compounds were first being synthesised from inorganic sources.

The minutest micrococcus visible under the microscope contains organic nitrogenous compounds in the form of proteins; it is probable that the ultra-microscopic particles, called filter-passers, which can pass through a Chamberland filter and yet can reproduce themselves in organic media, and transmit diseases, also contain organic nitrogenous substances. Their very specific reactions show these ultra-microscopic germs to be highly organised systems, on the path from the inorganic to the organic. It is hence futile to seek at the level of bacteria for the origin of the organic; the first stage must have been an inorganic colloidal solution-aggregate, capable of utilising sunlight and of building up on its surface or in its meshes organic substances as a forerunner for the still ultra-microscopic living cell. The powers of such a synthesising colloid in evolving more complex forms of matter would soon have been exhausted had it not been able to fix nitrogen as well as carbon from its environment. This power of nitrogen fixation, as evolution proceeded, must necessarily have been passed on to the more highly organised forms as these arose, for without such assimilative power they could not survive in a fresh world containing no ready-formed organic food.

Just as in carbon fixation, at a certain stage in evolution, a *luxus* method of fixation was evolved in the chloroplast of the green cell, which was able to work at a higher velocity and use longer wave-lengths of light than the simpler inorganic colloid which preceded it, so in the case of nitrogen, the slow union of nitrogen and oxygen to form oxides of nitrogen, induced by sunlight, in inorganic systems, became replaced by more rapid transformers in the unicellular green plant; and when organic matter began to accumulate, still another *luxus* channel was opened by those bacteria and other unicellular organisms which can use the supply of energy of decaying non-nitrogenous matter to build up nitrogenous compounds, utilising atmospheric nitrogen for the purpose. Examples of such cases of *luxus* fixation are the azoto-bacter living free in the soil and the nitrogen-fixing bacteria in the tubercles of the rootlets of the Leguminosæ. It is obvious, however, that there existed no decaying organic matter at the first dawn of the organic, and that Leguminosæ and all plants living in symbiosis with nitrogen-fixing organisms are late creations in the evolution of life.

Interesting and of great practical importance as are these symbioses of a carbon-fixing organism with a nitrogen-fixing organism subsisting upon it or upon its dead products, it is logically quite clear that they cannot have formed the origination of life and are only by-paths of evolution. One of the earliest organisms must have possessed the dual function united in a single cell, or solution-aggregate, of fixing both carbon and nitrogen. Approaching the subject from this aspect, we have examined the simplest unicellular algæ, and have found that they do possess this dual function.

In order to obtain as pure air as possible, the exposures and growths were made in the open air at Heron's Lodge, Heronsgate, Chorley Wood, Herts. The house is about 1½ miles from the railway line, stands on high ground at an elevation of about 400 feet, and possesses a large lawn, in the middle of which all the experiments, except the first, were made. The air was tested daily for nitrites by starch and potassium iodide papers, and nitrites were nearly always present. At the conclusion of the experiments, the growths and waters were taken to the laboratory of the Department, then situated at the Lister Institute, and there analysed for content of nitrogen by the Kjeldahl method. The experiments were made in the spring and summer of 1918, in the months of April to August inclusive.

Experiment 1.—Commenced April 15, finished August 5. We took two clear glass flasks of Jena glass, each of 1000 c.c. capacity, and provided with ground glass stoppers. These were almost completely filled with water from a clear stream near the house. One was filled up to the glass stopper, and then the stopper was inserted, so that the contents of the flask were completely shut off from the air. The other flask was filled so that the water stood in its neck about 4 cm. from the top; the glass stopper was not inserted, but instead a loose plug of cotton-wool was used. Over this a piece of fine muslin was placed and tied round the neck of the flask with thread; air, accordingly, had access to the contents of this flask. The glass-stoppered flask is a control to give the amount of nitrogen present at the outset plus any that can possibly be formed in absence of air.

The two flasks were placed outside on the ledge of a first-floor window, facing almost due west, so that direct sunlight was only obtained for a few hours in the afternoon. Care was taken to give equal chances to the two flasks by reversing their positions on the ledge every two or three days. Also a small beaker was inverted over the cotton-wool, to prevent contamination by rain. The flasks were not inseminated with any organism, but left to develop their crops naturally.

On May 2 a distinct green growth appeared in both flasks, slightly greater in the glass-stoppered one. On May 7 the open flask shows a distinctly greater growth than the stoppered one. In direct sunlight bubbles of gas are evolved from both, but most from the open flask. May 14.—Growth in the stoppered flask has stopped, no gas bubbles are given off in sunlight, but there is a brisk effervescence in the open flask, and the growth is obviously increasing. May 20.—All growth is stopped in the closed flask, and the growth has turned brown and flocculent. But the growth in the open flask is healthy and increasing, and gives off bubbles in sunlight. Frequent observations were taken till the conclusion on August 5, but for brevity these may be omitted. The growth in the open flask remained green and healthy to the end. No fresh growth started in the stoppered one, and at the end it had only a very few brown flocculi from the initial effort, ere its small store of dissolved carbon dioxide and nitrogen became exhausted. The contrast at the end between the two flasks was most marked.

Frequent examination with the microscope, through the wall of the flask, of the growing clumps of green organisms, showed the presence of only two types of round-celled unicellular algæ. One of these was smaller than the other and of a brighter green, and was identified as Chlorella; the other was of about twice the diameter, not so regularly spherical, and of a brownish-green colour. There were no Diatoms or other motile green organisms. No mycelium or other evidence of fungi were seen, and the absence of any turbidity at the end showed there was no appreciable development of bacteria. Drops examined at the end showed no bacterial growths.

Blank experiments on the water used showed that it contained about 1 mgrm. per litre of amino-nitrogen, and about 0·3 mgrm. per litre of nitrogen as nitrite and nitrate. In addition to this control, there is the control of the stoppered flask contents.

The contents of the two flasks were analysed on August 5. The water in each, slightly acidified with sulphuric acid to prevent any loss of amino-nitrogen, was evaporated down almost to dryness; then in each case the growth was added; destruction with sulphuric acid followed, and the nitrogen determination was made by the ordinary Kjeldahl's process.

The results were that the open flask contained 5·00 mgrm. of nitrogen, while the stoppered flask contained only 1·95 mgrm. A third flask of transparent silica, containing the same water, which had been exposed during the entire period of the experiment to such bright and direct sunlight as was available, developed no growth whatever; probably because the cells were destroyed by the intenser light and shorter wave-lengths. The water in this flask was examined similarly to the two above, and yielded 1·80 mgrm. per litre. There is thus a distinct and quite unmistakable gain of 3·05 mgrm., which has no other conceivable source than the atmosphere. It may be pointed out that this weight of nitrogen roughly corresponds to about 100 mgrm. of dried algæ, or to 500 mgrm., or about half a gramme, of moist-plant. It is an increase in nitrogen, lying many times outside the limits of error of the analytical methods employed.

Experiment 2.—Commenced May 31, 1918; terminated September 9, 1918. This experiment was carried out in a series of twelve screw-stoppered fruit preserving jars, called "Kilners," each of about 850 c.c. capacity. These jars are of pale-green bottle-glass, and possess a flat flange at top, over which is placed a flat rubber band; on this is placed the glass cover, provided with a flat flange to come in apposition. An air-tight union is obtained by means of a metal screw-top working on a glass screw-thread moulded on the outside of the wide neck of the jar. It was hence possible to keep certain of the jars in connection with the atmosphere by merely covering with a tied-on cap of lawn, and in others to screw down the lids and shut off access to the atmosphere. Each jar received 200 c.c. of tap-water, the supply being Rickmansworth water. This is a surface water containing a small amount of calcium salts, but otherwise a pure, good water.

Each jar was next inseminated with 2 c.c. of a dilute stirred up suspension in water of an algal growth which had been developed by self-growth in a small muslin-covered jar on the west window. The amount of nitrogen introduced in this insemination was infinitesimal, and, in any case, its amount, as well as any trace in the 200 c.c. of tap-water, can be accounted for by deducting the average small amounts of nitrogen found in Nos. 1, 3, and 4, which really serve as controls, the net increases in the others then show the favouring action on nitrogen fixation of the various procedures. After the addition of the 200 c.c. of tap-water and the 2 c.c. of algal, suspension in all twelve cases, each was next treated as noted in the schedule below, and afterwards they were either kept closed with glass lids screwed down, or covered with fine lawn only, and were kept in light or darkness as noted. Darkness was secured in a wooden shed close alongside. Light meant full exposure to daylight, on the middle of the grass lawn, except at mid-day in the hot weather, when a muslin shade was thrown over, and tin sheets placed loosely on top to protect from excessive light and heat, which are fatal to algæ.

In order to save space in tabulation the amounts of nitrogen found by the Kjeldahl determination carried out at the conclusion of the experiments are here placed opposite the description of the treatment of each jar:—

No. 1.—Tap-water and algæ only, glass lid screwed down, kept in light. Nitrogen = 0·3 mgrm.

No. 2.—Tap-water and algæ only, no glass lid, top covered by fine lawn, kept in light. Nitrogen = 1·6 mgrm.

No. 3.—Tap-water and algæ only, screwed down, no glass lid, kept in darkness. Nitrogen = 0·1 mgrm.

No. 4.—Tap-water and algæ only, covered by fine lawn, kept in darkness. Nitrogen = 0·3 mgrm.

No. 5.—Tap-water, algæ, 2 c.c. of 5 per cent. $Na_2HPO_4,12H_2O$ (*i.e.*, 0·05 per cent. of $Na_2HPO_4,12H_2O$) lawn cover only, kept in light. Nitrogen = 8·1 mgrm.

No. 6.—Tap-water, algæ, 0·05 per cent. of Na_2HPO_4 screwed down glass lid, kept in light. Nitrogen = 1·0 mgrm.

No. 7.—Tap-water, algæ, 0·05 per cent. of Na_2HPO_4, 0·05 per cent. of Na_2NO_2, lawn cover only, kept in light. Nitrogen = 12·3 mgrm.

No. 8.—Tap-water, algæ, 0·05 per cent. of Na_2HPO_4, 0·05 per cent. of NaNO2, screwed down glass lid, kept in light. Nitrogen = 0·7 mgrm.

No. 9.—Tap-water, algæ, 0·05 per cent. Na_2HPO_4, 0·05 per cent. $NaNO_2$, formaldehyde vapour at high dilution supplied from side tube as described below, glass lid screwed down, kept in light. Nitrogen = 3·8 mgrm.

No. 10.—Tap-water, algæ, 0·05 per cent. Na_2HPO_4, no $NaNO_2$, formaldehyde vapour, glass lid screwed down, kept in light. Nitrogen = 1·0 mgrm.

No. 11.—Tap-water, algæ, 0·05 per cent. Na_2HPO_4, 0·05 per cent. $NaNO_2$, dilute methylic alcohol vapour supplied from side tube, glass lid screwed down, kept in light. Nitrogen = 7·4 mgrm.

No. 12.—Tap-water, algæ, 0·05 per cent. Na_2HPO_4, no $NaNO_2$, dilute methylic alcohol vapour from side tube, glass lid screwed down, kept in light. Nitrogen = 1·1 mgrm.

Notes on Growth.—These were taken at frequent intervals and were concordant throughout, so as to save space only two samples will be given. It may be noted that in the course of growth the experiment was demonstrated to many scientific friends, and exhibits made at meetings of societies, such as the Bio-Chemical and Royal Microscopical Societies, and it was admitted that the differences in growth existed; this is confirmed by the nitrogen determinations now given at the conclusion of the experiment.

July 1, 1918.—No. 1, no growth; No. 2, slight green growth; Nos. 3 and 4, no growth; No. 5, growth in clumps; No. 6, one spot of growth only; No. 7, strong dark green growth all over bottom and sides, best of series; No. 8, slight growth; No. 9, slight growth, better than No. 8; No. 10, no growth; No. 11, fair growth; No. 12, no growth.

July 20, 1918.—No. 1, no growth; No. 2, fair growth, quite obvious difference between Nos. 1 and 2; Nos. 3 and 4 (in darkness), no growths; No. 5, good growth in thick discrete clumps; No. 6, only one colony commencing to degenerate; No. 7, the best of the series. Abundant dark green growth and many gas bubbles; No. 8, fair growth, but distinctly less than in Nos. 7, 9, 11; Nos. 9 and 11, both show three or four times as much green growth as No. 8; No. 10, no growth; No. 12, very slight growth.

At the end, just before Kjeldahling, a microscopical examination was made of Nos. 5, 7, 9, 11. All these showed almost pure growths of small round single green cells (Chlorella); there were no mycelia or spores or other types of non-green cells.

Commentary.—Several distinct facts are shown by this experiment, and taking the final amount of nitrogen as quantitative guide, these will now be pointed out. It is interesting in the first place to notice the increase in nitrogen which could only come from the atmosphere in those cases where no source of nitrogen was added from without, but other essential conditions of growth were more or less perfectly satisfied. It is next of importance to note the effects of limiting each essential factor in turn, namely, carbon dioxide, easily available nitrogen (nitrites), alkaline phosphate, and to note the great swing in photo-synthesis in No. 7, where all are adequately satisfied. Lastly, the important proof is clearly furnished, we believe, for the first time, that carbon dioxide can be completely cut off if its place be supplied by simple organic substances, such as formaldehyde and methylic alcohol, which have hitherto been regarded as the first products of photo-synthesis in the green cell, on purely circumstantial evidence, or upon very weak and uncertain positive experiments.

The uptake of nitrogen from the atmosphere is shown by the difference between Nos. 1 and 2 with nothing whatever added, only tap-water and a slight insemination with the algæ present, the sole difference being that No. 1 is screwed up airtight, while No. 2 is open to the air. The difference in nitrogen-content, $1 \cdot 6 - 0 \cdot 3 = 1 \cdot 3$ mgrm. is quite unmistakable. The limiting factor to growth here is unquestionably deficiency in phosphate in the tap-water, as is clearly proven by Nos. 5 and 6, where this defect is supplied. This experiment again in a still stronger way shows the absorption of nitrogen from the air.

The fillip of the phosphate in No. 6, with screwed-down lid, has caused a light commencing growth until carbon dioxide became exhausted, amounting to 1 mgrm.; but when this is contrasted with No. 5, differing only in access of air, *i.e.*, available carbon dioxide and nitrogen, the figure runs up to 8·1 mgrm., an increase of 7·1 mgrm. Now taking this No. 5 as it stands by itself, and not contrasting it with any of the others, the proof is given that in 200 c.c. of tap water, plus 2 c.c. of 5 per cent. solution of di-sodium hydrogen phophate, *plus* a minute insemination of Chlorella, there is an abundant growth, and the absorption from the air of 7 to 8 mgrm. of nitrogen. The controls are abundant and the conclusion seems to us inevitable.

Next observe the effects of a *luxus* addition of easily available nitrogen as nitrite, as shown by No. 7. This differs from No. 5 only in the extra addition to the culture medium of 0·05 per cent. of sodium nitrite, and the crop goes up by 50 per cent. from 8·1 to 12·3 mgrm. Similarly in the effect produced by manuring with ammonium salts or nitrates, a luxury supply produces a *luxus* crop.

The small growth in No. 8 with lid screwed down shows that available phosphates and sources of nitrogen may be present, but when the supply of carbon dioxide is cut off, and no substitute supplied, no photo-synthesis can occur. This is obvious, but No. 8 is a necessary control to No. 7 where CO_2 is supplied from the air, and to Nos. 9 and 11, where the air is shut out, but a supply of excessively dilute vapours of formaldehyde and methylic alcohol respectively are obtained from these substances contained in narrow test-tubes placed slantingly in the jars before the lids were screwed down. The result demonstrates that supplied in this manner the vapours of these simple organic substances can substitute carbon dioxide quite well, there being 3·8 mgrm. of nitrogen with the formaldehyde vapour and 7·4 mgrm. with the methylic alcohol, as against 0·7 mgrm. in the control. This does not show quantitatively that methylic alcohol is better as a nutrient than formaldehyde, for with somewhat increased concentrations both vapours would kill the growing green cells, and it is a difficult problem so to regulate the concentration of each at these excessive dilutions that the maximum beneficial result is obtained. The real point is that these vapours of simple organic substances, when sufficiently dilute, act not as poisons to the green cell but as nutrients which can replace carbon dioxide.

The bearing of these results upon the fundamental hypothesis of Baeyer, that the first step in the synthesis in green cells, of the organic from the inorganic, is formaldehyde, is so intimate that a little more consideration appears desirable. Hitherto this hypothesis has rested mainly upon circumstantial evidence. No one has ever been able to demonstrate the presence of formaldehyde in the living cell. Various solutions of chlorophyll and emulsions of chlorophyll in water, or upon colloidal membranes, such as gelatine, have been exposed to light, and afterwards formaldehyde has been tested for by delicate chemical tests. The results have been contradictory; some observers have found formaldehyde and others have failed to detect it. But we have previously shown that practically any organic system, when exposed to light, yields formaldehyde. Hence the presence of formaldehyde under such conditions means nothing and probably comes from a reversed system, not of building up but of breaking down of organic substances. Even if formaldehyde could be shown intra-vitally in a living cell it might only mean excessive light exposure and breaking down of living substance and not building up. We have pointed out the same in previous communications (3, etc.), viz., that the lethal action of light upon bacteria and other living cells is probably due to such a reversed reaction, in which formaldehyde and other simple and poisonous organic substances are set free.

The other channel of approach experimentally to this question is more promising, namely, Can these simple organic substances act as nutrients for cells? If formaldehyde be the primary stage in photo-synthesis by which green cells are nourished, then it ought to be possible by supplying formaldehyde when carbon dioxide is shut off to make green cells grow and flourish.

Many attempts have been made in this direction, but have all failed, or given very dubious results, because of the highly poisonous action of formaldehyde. Formaldehyde, if and when formed in a green cell, must immediately be condensed into a sugar or some other non-poisonous organic compound or the cell will perish; there is, accordingly, no demonstrable amount of formaldehyde in the cell. If now it be supposed that the cell in sunlight is always producing formaldehyde which at once is changed into something else, then in order to mimic this process experimentally a system must be invented in which formaldehyde at minute concentrations is fed in slowly, at a rate not greater than the cells of the system can assimilate it. The formaldehyde must not be added to the solution in which the cells are growing at the outset, for any quantity detectable afterwards by increase in the cells would kill them. It must be continuously and very slowly administered as a dilute vapour.

Two glass tubes of about 0·5 cm. in diameter, each about 18 cm. long, were sealed at the one end so as to make narrow test-tubes. One of these was about half filled with formol (40 per cent. formaldehyde), the other with methylic alcohol. The tube containing formol was placed slanting in bottle No. 9, the closed end resting on the bottom of the bottle, and the upper end on the inside of the neck close beneath the glass lid. The tube containing methylic alcohol was similarly placed in No. 11. Both glass lids were tightly screwed down to exclude atmospheric carbon dioxide. The nutrient solutions contained nitrite as well as phosphate. The two flasks were kept exposed to light, and after some weeks there were good growths obtained in both.

Judged from the amounts of nitrogen there was a fixation of 3·5 mgrm. in the formaldehyde nutrition, and a fixation of 7·1 mgrm. in the methylic alcohol nutrition. To get from the nitrogen fixation to the carbon fixation these figures must be multiplied by a factor of at least 8, for the weight of carbon even in protein is treble that of the nitrogen, and there is carbon but no nitrogen in carbohydrates and fats. If this factor be applied there is a fixation of 28 mgrm. of carbon from formaldehyde (= 70 mgrm. of formaldehyde), and of 56·8 mgrm. of carbon from methylic alcohol (= 151 mgrm. of methylic alcohol). The bottles Nos. 10 and 12 were a similarly conducted experiment, but without available nitrates or other easily assimilable nitrogen, and this has greatly repressed the production of growth. The two amounts of nitrogen of 1·0 and 1·1 mgrm. are, however, considerably above the controls, namely, 0·1 to 0·3, so that it would appear probable that there was a slow fixation of atmospheric elemental nitrogen. (See in this connection the next experiment, Nos. 3 and 4, and the succeeding paper on Marine Algæ.)

Experiment 3.—Commenced August 9, 1918; terminated August 29, 1918. This set was likewise carried out in Kilner jars, and was designed to test whether, with exclusion of the free atmosphere, nutrition and growth could be achieved by feeding with high dilutions of carbon dioxide, and of oxides of nitrogen. The experiment was carried out as follows:—

Eight Kilner jars were taken. Each of these received 100 c.c. tap-water, 1 c.c. of a 1 per cent. solution of sodium chloride, 1 c.c. of a 1 per cent. solution of alkaline potassium phosphate (K_2HPO_4), and two drops of a 1 per cent. solution of ferric chloride (equivalent to 0·14 c.c.). Then to each was given a minute insemination of 1 c.c. of a dilute suspension of a unicellular green algal growth. The eight jars were set out on the lawn in the daylight on August 9, each being loosely covered by its lid, but opened daily, so as to establish growth in each case preparatory to the subsequent treatment. On August 16 growths are evident, and about equally advanced in all the jars; numerous fresh colonies have started attached to the bottoms, and in the precipitated calcium phosphate. The eight jars were now divided into four pairs. The first pair (Nos. 1 and 2) simply had their lids screwed on airtight. The second pair (Nos. 3 and 4) first had a narrow test-tube, similar to that described in Experiment 2, and half filled with solid sodium bicarbonate inserted, and both then had their lids screwed on airtight. The third pair (Nos. 5 and 6), instead of the sodium bicarbonate tube, had a similar tube, containing a system designed to evolve nitrogen peroxide and other oxides of nitrogen very slowly into the air of the jar. The fourth pair (Nos. 7 and 8) each had two tubes, one containing sodium bicarbonate, and the other the nitrous system. This nitrous system was constructed thus:—A few crystals of sodium nitrite were introduced into the narrow test-tube, the tube was then gently half filled with water, and on top of this there was introduced a column of about 3 cm. of 1 in 10 nitric acid.

The design of the experiment was to shut off all atmospheric supply of carbon dioxide and of nitrites, and in one pair leave the algæ destitute of both these; in the next, supply carbon dioxide and restrict nitrites; in the third, supply nitrites but restrict carbon dioxide; and, in the fourth, supply both carbon dioxide and nitrites. The dissociation pressure of carbon dioxide from sodium bicarbonate lies between 1 mm. and 2 mm. of mercury, so it is ample to supply the needs of the algæ; also the nitrous system was so arranged as to give off the nitrous fumes at such a rate that the algæ could cope with them and utilise them, so that the nutrient medium did not become acid.

Examined on August 30 (*i.e.*, 14 days after the addition of the side-tube), it is found that Nos. 1 and 2 (no addition) and Nos. 5 and 6 (nitrites only) are dead and degenerating. This had occurred in about a day in Nos. 5 and 6, probably because they could not grow from lack of carbon dioxide, and so the oxide of nitrogen absorbed by their culture media accumulated and killed them. Degeneration did not occur in Nos. 1 and 2 for about a week to ten days, but at the end of the fortnight they were obviously dead and degenerating.

The contrast in the case of the other four jars was striking; all four had lived, increased in amount, and were flourishing at the end. It was obvious that the pair which had received both carbon dioxide and oxides of nitrogen were much in advance of the other pair, which had been supplied with carbon-dioxide only. The latter, of course, had a supply of elemental nitrogen in the enclosed air of the jar.

The united contents of each pair of jars, when Kjeldahled, gave the following results:—

	Nitrogen.
Nos. 1 and 2 (no additions)	3·46 mgrm.
Nos. 3 and 4 (sod. bicarb. only)	5·40 „
Nos. 5 and 6 (oxides of nitrogen only)	2·50 „
Nos. 7 and 8 (both sod. bicarb. and oxides of nitrogen)	14·10 „

The larger weight of nitrogen found in Nos. 5 and 6, which did not grow after addition of oxides of nitrogen, as compared with the controls of the previous experiment, arises from the growth during the first week open to the atmosphere, when the growths were allowed to strike; no such period was allowed in the preceding experiment. If the amount of 2·50 mgrm. in this pair be taken as the control, then the amount of nitrogen fixed by jars, with no additions, before succumbing to lack of carbon dioxide is 0·96 mgrm. When carbon dioxide is supplied from the sodium bicarbonate, but no oxides of nitrogen are given to the air, so that the only source for the fixation of nitrogen* is the elemental nitrogen of the air enclosed in the jars, this figure rises to (5·40 − 2·50) 2·90 mgrm. This result is of high importance, for it shows that, given supplies of carbon dioxide, nitrogen, and oxygen, in presence of sunshine, the cell can form its own oxides of nitrogen and build these into amino-compounds, although at a much slower rate than when oxides of nitrogen are supplied.

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* As we have shown in a previous paper, this air contains a trace of nitrites, but the amount in the few hundred cubic-centimetres of air contained in the jar is infinitesimal and may be neglected.

This is confirmed again by the experiments on marine algæ to be detailed in the succeeding paper. In the sea-water a source of carbon dioxide already exists in the bicarbonate of magnesium and calcium dissolved in it. Hence no side-tube is necessary, and a marine alga simply shut up airtight photosynthesises and fixes both carbon and nitrogen. The stimulating and growth-quickening effect of traces of oxides of nitrogen, passing from the air to dissolve in the aqueous medium bathing the green cell, is shown by the great rise when these oxides are supplied, as in Nos. 7 and 8.

The amount of nitrogen here fixed is 11·60 mgrm., as compared with 2·90 when elemental nitrogen is the sole source.

Summary.

1. The primæval living organism, like the inorganic colloidal systems which were its precursors, must have possessed the power of fixing carbon and nitrogen and building these up into reduced organic compounds with uptake of energy. The source of the energy was sunlight.

2. This power is still possessed by the lowliest type of synthesising cell existing, namely, the unicellular alga.

3. A synthesising cell must have existed prior to bacteria and other fungi, since these can only exist upon organic matter, and the primæval world before the advent of life could contain no organic matter.

4. Their specific reactions show that even the ultra-microscopic filter-passing organisms are highly organised products on the path from the inorganic towards life, and it hence follows that there is a long intermediate range of evolution. The first synthesising system acting upon the light was hence probably an inorganic colloidal system in solution, capable of adsorbing the simple organic substances which it synthesised. It is hence futile to search for the origin of life at the level of bacteria and torulæ.

5. As complexity increased with progressive evolution more and more rapid transformers for the capture of the energy of the sunlight came into existence. Such transformers are found in the green cell for fixation of both carbon and nitrogen. The earlier transformers in the inorganic colloidal systems can only utilise light of short wave-lengths; the later transformers in the living cells are adapted to utilise longer wave-lengths, and the very short wave-lengths, which are lethal, are cut off by their colour screens of chlorophyll, etc.

6. The earliest products of photo-synthesis, such as formaldehyde and methylic alcohol, are highly poisonous to the green cell; but fed to it at sufficiently high dilution, can be used as nutrition in absence of carbon dioxide, and very marked growths have been obtained with these substances as the sole source of carbon.

7. In the absence of all other sources of nitrogen save the elemental nitrogen of the atmosphere, but with abundance of carbon dioxide, the unicellular algæ can fix nitrogen, grow and form proteins.

8. The rate of fixation and growth is, however, greatly accelerated if nitrites or oxides of nitrogen are available.

9. These oxides of nitrogen can be supplied in gaseous form from the amosphere, and pure country air normally contains such oxides of nitrogen, especially in spring and summer.

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A Preliminary Account of the Meiotic Phenomena in the Pollen Mother-Cells and Tapetum of Lettuce (Lactuca sativa).

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(Communicated by Prof. J. B. Farmer, F.R.S. Received March 1, 1920.)

On examining some lettuces which Prof. William Bateson, F.R.S., was growing in his experimental grounds at Merton, I was struck by the differences between a certain Cos lettuce (Sutton's "Dwarf Perfection") and a rogue from it which showed some resemblances in leaf characters to a cabbage lettuce. Prof. Bateson kindly permitted me to collect some cytological material of the variety and its rogue, and a subsequent study of the pollen development, particularly in the variety, has developed several points of so much general interest that a preliminary account of certain features was deemed desirable. So far as the work has progressed no constant difference between the variety and its rogue has yet appeared, the chromosome number being the same in both. The present account, so far as known, applies equally to the variety and the rogue. Only the main points of special interest in the meiotic history will be touched upon in this communication.

The material was fixed in various chrom-acetic acid solutions, some of which gave very good fixation, and the sections were chiefly stained with Heidenhain's iron-alum hæmatoxylin. In the resulting preparations the structural features both of the chromatin and the cytoplasm were beautifully clear, the presence of latex tubes causing no difficulties and requiring no special treatment.

One of the striking features first observed was the variety of conditions in the tapetum, all transitions between pollen mother-cells and tapetal cells being found. This was all the more unexpected as occurring in a composite, and since the Compositæ stand high among flowering plants the condition can scarcely be regarded as a primitive one. The tapetal cells frequently become greatly elongated and are in some cases very narrow, but they lie almost invariably with their long axes lengthwise of the loculus. They become binucleate by a mitotic division of the nucleus about the time of synapsis in the pollen mother-cells, and many of them later become tetranucleate by another mitosis.

These tapetal cells vary enormously in size, both in the binucleate and the tetranucleate conditions. The chromatin of the nuclei, particularly in the binucleate cells, in certain cases shows appearances which are indistinguishable from certain synaptic stages of the nucleus. In all such cases the two nuclei of the cell are in exactly the same condition. The typical synaptic knot or synizesis has been observed very few times in tapetal nuclei, but in many other binucleate cells the condition of the nuclei very closely resembles the post-synaptic stages in the pollen mother-cells. It is, therefore, very difficult to say whether certain binucleate cells should be regarded as pollen mother-cells which have become binucleate and yet are continuing the post-synaptic nuclear phenomena of meiosis, or whether they should be regarded as tapetal cells which are peculiar in showing these nuclear conditions. In any case it is clear that all transitions between pollen mother-cells and tapetal cells occur, and that such intermediate cells will ultimately break down and contribute to the tapetal plasmodium which is formed at a late stage of pollen development. It should be stated that in binucleate cells whose nuclei are in synizesis, the cytoplasm is well fixed and shows little or no contraction.

The phenomenon of synapsis was first recognised by Moore (1895) in Elasmobranch spermatogenesis, and its significance was further elaborated in the classical paper of Farmer and Moore (1905) on Meiosis. It has since been found as a practically universal preparation stage for the meiotic divisions in plants and animals, and is therefore coextensive with sexuality itself. This contracted condition of the nucleus was formerly believed to bring about the pairing of maternal and paternal chromosomes or elements. But since it has been shown that the paired arrangement of the chromosomes frequently occurs already in somatic divisions and probably in many cases comes about at the time of fertilisation, some deeper physiological significance of the contracted phase of the chromatin must be sought. An indiscriminate interchange of corresponding chromatin elements (chromomeres) was also formerly supposed to take place at this time between the delicate parallel threads. But all direct evidence of such a process has broken down, and the indirect evidence is strongly against its occurrence. Since the evidence for an interchange of particles has disappeared, writers have frequently spoken of an "influence" exerted upon each other by homologous chromomeres while they are in close approximation. It is difficult to see in what such an influence can consist. The more recent studies of synapsis by Wilson (1912) and others have failed to penetrate more deeply into the meaning of this contracted phase. Its occurrence in living cells shows that it is in no sense an artefact, but it must be admitted that no adequate explanation of its significance is yet forthcoming. Its unique occurrence at but one point in the life cycle however, emphasises its importance as a preliminary to meiosis, although a second contraction, after the spireme thread has attained the thickness of the definitive chromosomes, has now been described in many plants and in certain animal forms.

So far as I am aware, the synaptic contraction has never before been observed, except in the spore mother-cells or corresponding structures of plants, and in the primary spermatocytes and oocytes of animals. Its occurrence as a rarity in the binucleate tapetal cells of lettuce is probably to be attributed to the fact that this plant shows the unusual condition in which all transitions between tapetal and germinal cells occur. Its presence here under these circumstances is not surprising, and may perhaps lead to a physiological interpretation of the phenomena of synapsis. One is tempted to think of it as a plasmolysis of the nucleus, or rather of its contents. But it is evident that such a simple explanation will not suffice.

In a detailed study of the history of the tapetal nuclei in certain Angiosperms, Bonnet (1912) suggested that the two meiotic divisions leading to the tetranucleate condition corresponded to the two meiotic divisions in the pollen mother-cells. But neither of these divisions in the tapetal cells is ever a reduction division so far as known, nor are they preceded by synapsis of the nucleus. Moreover, the synapsis which has been observed as an exceptional occurrence in lettuce comes in the *binucleate* condition of the tapetal cells, after the first mitosis has taken place. This appears to emphasise its physiological aspects as a reproductive process, and leads one to look upon synapsis (or more specifically synizesis), as a phenomenon of the nucleus which might be directly induced in any diploid cell if it could be placed under the proper conditions.

Another phenomenon which may occur in *Lactuca sativa* when the nuclei are in synapsis, is the process of chromatin extrusion which I have elsewhere (Gates, 1911) called cytomyxis. It has been found only rarely in lettuce, but when it occurs it has the same characteristic features observed in other plants. So far as known, it has not been observed in animal spermatocytes, and the structural conditions of the primary spermatocytes are such that it would not be expected to take place.

This extrusion from the pollen mother-cells only takes place when the nucleus of a pollen mother-cell in synizesis becomes so eccentric in position that its membrane comes into contact with the cell wall separating it from the next pollen mother-cell. If, under these circumstances, the nuclear membrane happens to touch the cell wall at a point where an opening or cytoplasmic connection with the next cell exists, then a portion of the viscous chromatin will flow through into the cytoplasm of the adjacent cell. Presumably a nucleus which has thus lost a portion of its chromatin will not be able later to complete its normal development. There is, however, no clear evidence that the process is an artefact, though it has often been considered such. Cytomyxis has now been described in a number of forms, always occurring in the pollen mother-cells during synapsis. The literature of the subject will be considered when the complete account is published.

Synapsis in the pollen mother-cells in lettuce is followed by the usual spireme stages ending in diakinesis, which will be fully described, with their variations, in the final paper. There are nine pairs of chromosomes in diakinesis, and in size the definitive chromosomes form a graded series, two or three being of maximum length (several times their diameter), three or four of intermediate length (about twice their diameter), and the remaining three or four nearly isodiametric. In width there appears to be no constant difference, although there is considerable variation. The pairing of the chromosomes in diakinesis is exceptionally close for plant material, the members of a pair almost invariably lying parallel, and the pairs giving no evidence of their bivalent nature, except occasionally by a split at one end or a light line down the centre. There is great variation in the bulk of the chromosomes in different mother-cells, but the size relationships of the chromosomes within a nucleus appear always to be maintained.

In the earlier stages of formation of the diakinetic chromosomes, before they have begun shortening and thickening, their bivalent nature is very clearly apparent. The two members of a pair lie as long threads of chromatin side by side, or in many cases wrapped round each other in various ways. They may be looped in the middle to form a figure 8, or there may be two or even more loops in the length of a pair of chromosomes. In other cases one chromosome is more nearly straight, while the other is wrapped round it. In some cases the fusion of the loops at the points where they cross each other, appears so intimate that they are likely to break apart with an interchange of segments. This course of events, known as chiasmatypy, was described by Janssens (1909) in Batrachoseps, and has been used by Morgan (1915) as the probable physical basis of the crossing-over phenomena of heredity which he and his pupils have described with a great wealth of detail in the fruit fly, Drosophila. So far as I am aware, the phenomenon of chiasmatypy is here described for the first time in plants, although twisted chromosome pairs have been figured in various forms. Its full description, with figures, is reserved for the final paper.

It is an interesting fact that in these stages, during which the diakinetic chromosomes are being formed, some pairs may shorten and thicken much more quickly than others, assuming their definitive shape while the others still remain as long and narrow threads. The same phenomena has been described in Oenothera (Gates, 1908).

In the study of large numbers of pollen mother-cells in diakinesis, occasional departures from the normal nine pairs are found. In one such case two of the longest chromosomes had obviously fused together end to end, forming one enormously long chromosome. The fusion was complete, and there was not the slightest indication of a line of separation between them. This nucleus, therefore, contained eight separate chromosome bodies, the other seven pairs being unaltered. In certain other nuclei where only eight bivalent chromosomes were present, there had probably been a fusion of two pairs, but it was not possible to identify with certainty which pairs had amalgamated. In at least one case it would appear that some other process than fusion may have brought about the diminution in number. Ten chromosome bodies were found in diakinesis in a limited number of cases. Whether they have arisen through a transverse segmentation of one of the bivalent chromosomes or by the separation of the members of a pair can perhaps not be determined with certainty, owing to variations in chromosome width. In any case, however these alterations come about they can be looked upon as temporary or permanent germinal changes according to their later behaviour. They serve merely to emphasise the remarkable constancy in the history of the chromosomes in the germ-cell cycle. Probably all organisms will show such occasional alterations when large numbers of their germ cells in the same stage of their development are critically studied and compared.

Another phenomenon of much interest in the meiotic chromosomes of lettuce is the tendency which appears for certain of the bivalent chromosomes to coalesce more or less completely on the equatorial plate of the heterotypic spindle. At this stage the chromosome pairs become even more condensed than in the late diakinesis, assuming finally an almost globular shape. They frequently show at this time their point of attachment to the spindle, as a thread-like prolongation from one end of the chromosome. All indications of their bivalent nature have now completely disappeared. In counting the chromosomes at this stage, either in polar view or in side view, I was at first able to find only seven bodies. It was necessary, of course, to assure oneself that such chromosome groups were uncut in sectioning. Then cases were found where two of the bivalent chromosomes were more or less completely coalesced, and, finally, cases in which all nine could clearly be counted. The diminution in number at this time is thus found to be due to the temporary coalescence of two bivalent chromosomes, probably end to end, giving eight bodies, or of two more, diminishing the number to seven bodies.

So far as I know, the literature of cytology contains no similar instance of chromosome behaviour, either in plants or animals. It can hardly, I think, be ascribed to the fixation, for it occurs in varying degrees in cells closely grouped on the same slide, and where there is every indication that the fixation is good.

There are indications that, in some cases at least, the shorter chromosome bivalents are the ones which coalesce on the equatorial plate of the heterotypic spindle, though it is difficult to identify them with certainty at this time owing to their extreme condensation. Nor is the process by any means a uniform or invariable one. There is rather a *tendency* towards the coalescence of one or two pairs. In many cases the full number nine can be clearly counted in this metaphase stage. In other cells, particularly when the differentiation of the chromosome stain has gone rather far, the partial coalescence of particular pairs can be clearly determined. In still others, the coalescence is so complete and intimate that only seven bodies can be counted in the heterotypic metaphase, either in polar view or side view.

There is clear evidence that, in some cases at least, and presumably in all, the coalescence of the bivalent chromosome has been end to end. Since the maternal and paternal members of each chromosome pair are indistinguishable from each other, it is impossible to know precisely how this longitudinal coalescence takes place. Whether, for example, the two paternal chromosomes always undergo an end to end fusion with each other (fig. 1), or whether there is an equal or greater chance of each paternal chromosome lying end to end with a maternal chromosome (fig. 2).

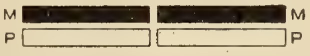

FIG. 1.

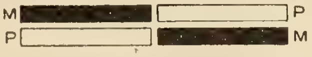

FIG. 2.

Assuming, as many lines of evidence go to show, that differences, which are inherited in Mendelian fashion, are determined by the presence of different chromosomes, we evidently have, in this temporary coalescence, the basis for disturbances in the chromosome distribution, and hence in the Mendelian ratios, with the appearance of what is called partial coupling or repulsion between factors. Such a result would only be absent in case the chances were equal that the end to end coalescence of the pairs would be between a paternal and a maternal chromosome, or between the two paternal and the two maternal chromosomes. There is no evidence that such coalesced chromosome pairs will pass over bodily to one pole of the spindle. Rather, they will both split in the usual way, but the manner of their coalescence will determine the nature of their distribution, whether, *e.g.*, the paternal halves of each chromosome will go to the same pole or opposite poles of the spindle.

This subject need not be discussed further here, but it evidently furnishes a possible basis for the phenomena of partial coupling or repulsion, apart altogether from the "crossing over" phenomena, which are based on relations between the two members of a pair of chromosomes in their earlier post-synaptic stages. In any case, the frequency, one might almost say regularity, with which this phenomenon of temporary coalescence of certain pairs of chromosomes takes place, indicates that it must have some special significance. While the coalescence usually occurs on the heterotypic spindle, it may in rare cases take place earlier, in diakinesis. But the earlier fusion, when it occurs, presents certain differences, and may represent a separate phenomenon.

Among other phenomena of interest in this study is the method of division of the pollen mother-cells into a tetrad after the meiotic divisions are completed. So far as observed, this process takes place, at least in the great majority of mother-cells, by invaginations of the cytoplasm developing between the nuclei at four equidistant points on the periphery, and gradually cleaving the cytoplasm into four cells. In many cases this process begins while the spindle fibres are still present connecting all the nuclei. But it occurs in spite of the spindles, not with their aid, and it can take place equally well in their absence. No case has yet been found in which cell walls are laid down on a spindle plate after the usual fashion. Although such a nuclear plate (non-functional) is occasionally seen as a temporary structure on the spindle in the heterotypic telophase, it has never been observed in the homotypic telophase, the cell division taking place only, so far as observed, by a constriction of the cytoplasm, either in the presence of the spindles or after they have disappeared.

As mentioned earlier, a tapetal plasmodium is ultimately formed, a very limited number of pollen grains maturing in the loculus and becoming surrounded by a mass of cytoplasmic detritus from the tapetum, in which cell walls have completely broken down, and the nuclei have almost or quite disappeared. This, again, is considered an uncommon condition in Angiosperms, although a plasmodial tapetum as a normal occurrence has been described in a number of forms (Juel, 1915).

In this brief preliminary account of the meiotic phenomena in lettuce, I have only touched upon some of the points which have appeared of more general scientific interest. A very detailed study, particularly of the history and variations of the chromosomes, has been made. It is considered that the study of variations in the behaviour of the chromosomes has been too much neglected, and that any account is incomplete which does not include a record of the conditions rarely or infrequently observed, as

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well as of those which seem to fall into the usual order of events. Doubtless, some organisms show much greater variation in these respects than others, and this also is not without its significance for genetics.

In connection with this work, I am greatly indebted to my research assistant, Miss E. M. Rees, B.Sc., the accuracy of whose drawings has added a great deal to the value of the results.

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On the Development of the Auditory Apparatus in Sphenodon punctatus.

By F. J. Wyeth, M.A., B.Sc., B.D.

(Communicated by Prof. Arthur Dendy, F.R.S. Received November 4, 1919.)

(Abstract.)

This memoir contains a detailed and fully illustrated account of the development of the auditory apparatus and associated structures in the New Zealand Tuatara. As this important type is on the verge of extinction, it was thought desirable to treat the subject fully, although, as might have been expected, the developmental history agrees closely with that found in other reptiles.

The work was carried out chiefly by means of wax plate reconstruction models. The development of the pharynx and its derivatives, the thyroid and thymus glands and the trachea, was investigated and appears to follow the normal course. The first visceral cleft undergoes some closure from below upwards and the process is completed by the apposition of the anterior and posterior borders of the cleft. The second and third visceral clefts are closed by a backwardly-growing operculum, while the fourth visceral pouch is imperforate and bears a rudimentary fifth visceral pouch as an evagination of its posterior surface.

The apparent absence of separate dorsal and ventral divisions of the visceral clefts whereby all or any of the first three pairs of visceral pouches communicate with the exterior would seem to be an indication of the primitive nature of this reptile. With this possible exception, the present observations do not reveal any abnormalities in the developmental changes undergone by the pharynx or by such of its derivatives as have now been considered.

The existence of two pairs of head cavities was confirmed, those of each pair communicating with one another by transverse canals. The head cavities appear to originate by the splitting of a single pair of cavities, and, at a later stage of development, there appear—in the regions formerly occupied by them—rounded mesoblastic masses which may be "premuscle masses." It is suggested, therefore, that each head cavity should be regarded as a "myocœle," but that the head cavities represent the cavities of the head somites is uncertain, as is also their suggested connection with the orbital muscles.

The vascular system was found to exhibit a number of primitive features. The arteria basilaris originates as a pair of completely separated vessels, and

the progressive fusion subsequently undergone by these primitive arteries supports the suggestion advanced by Dendy (1909) that the (incompletely-fused) condition of the arteria basilaris observed by him in adult Sphenodon is reminiscent of an earlier paired condition, and is therefore a primitive feature. The development of the persistent ductus arteriosus and ductus caroticus, the relationship of the arteriæ laryngealis, pulmonalis and cervici muscularis, and the progressive reduction and ultimate fate of the aortic arches have all been investigated. It has also been shown that Sphenodon is similar to other reptiles in respect of the persistence and primitive nature of the arteria stapedialis, which is derived from a persistent dorsal vestige of the second aortic arch. The presence of a number of newly-identified branches of this artery (the arteriæ tympanica, dentalis inferior, temporalis posterior and auricularis posterior) has also been recorded. As regards the venous trunks of the auditory region the most conspicuously primitive features are the persistence of the tenæ cephalicæ, mediæ and posteriores and their relationship to the vena capites lateralis and to the primitive head-veins of the early embryo. The presence and development of paired venæ faciales, tympanicæ, and cochleares have now been recorded for the first time.

It is concluded that the arrangement of the blood vessels in the auditory region of Sphenodon is similar to, but more primitive than, that found in Lacertilia.

The region investigated includes cranial nerves vi—xii, the development of which was worked out in detail. The primitive facial nerve early shows differentiation into one pre-trematic and one post-trematic branch. The former gives rise to the ramus palatinus, while from the latter develop the ramus communicans interna, the chorda tympani and the ramus hyomandibularis. From the last-named trunk spring the delicate ramus recurrens and also the ramus communicans externa which passes ventral to the stapes and, ultimately uniting with the ramus communicans interna, enters the ganglion petrosum.

The development of nerves vi, ix, x and xi presents no abnormal features. Within the foramen jugulare the roots of nerves ix, x and xi enter an incompletely-trilobed ganglion of which the three component bundles of fibres are clearly differentiated and between which the passage of few communicating fibres can be observed. The roots of nerve xii undergo, as development proceeds, a progressive reduction in number from five to two, the foramina by which they emerge from the exoccipital region of the parachordal plate being similarly reduced in number from four to two.

The general development of the inner ear and auditory nerve is thoroughly normal. The auditory organ appears as a nearly circular, and slightly hollowed patch of single-layered epiblast which rapidly develops into a deep auditory pit. Rapid upward growth of the ventro-lateral border of the pit ensues, the auditory pit being converted into an auditory sac (otocyst), the lumen of which communicates with the exterior by a narrow primitive ductus endolymphaticus.

The distal portion of the acustico-facialis neurencytium, by which the sensory end-organ was connected with the hind-brain, undergoes a simultaneous progressive differentiation into a dorsal or auditory and ventral or facial ganglionic rudiment, still united proximally to the hind-brain by a common root. The superficial area of the otocyst undergoes rapid expansion and it soon becomes constricted into a pars superior and a smaller pars inferior. The constriction passes through the large patch of neuroepithelium (primitive auditory epithelium)—which now occupies nearly the whole of the medial and a portion of the ventral wall of the otocyst—and divides it into two parts, each of which is connected with a portion of the auditory neurencytium.

The subsequent further division of these primitive sensory epithelial patches into the various maculæ acusticæ is accompanied by a corresponding division of the distal portion of the auditory neurencytium, which thus ultimately gives rise to the various ramuli of the auditory nerve. The present investigation confirms the suggestion that "the breaking up of the primitive auditory ganglion is a necessary accompaniment of the process of resolution of the sense-epithelium patch into its various macular areas" (Cameron and Milligan, 1910). The final closure of the otocyst takes place just dorsal to the middle of the lateral surface of the developing ductus endolymphaticus, and a protrusion of the medial surface of the same region now occurs, thus producing the rudiment of the saccus endolymphaticus. It is noteworthy that a vestige of the distal portion of the ductus endolymphaticus, now separated from the superficial epiblast of the head, persists in the oldest Sphenodon embryos examined, in which it occurs as a conical prolongation of the dorsal region of the saccus endolymphaticus situated in a foramen sacci endolymphatici piercing the cranial roof of either side, not far from the mid-dorsal line. This is obviously a primitive feature and has not been recorded in any of the higher Vertebrata.

The aperture by which the lumen of the ductus endolymphaticus communicates with that of the otocyst now undergoes displacement in a medioventral direction, owing chiefly to the expansion of the surface of the latter in a dorsolateral direction, although the process appears to be due, in part, to a ventral elongation of the closed portion of the ductus, which results from a progressive lateral fusion of its anterior and posterior walls, thus shutting off more and more of its lumen from that of the otocyst.

Simultaneously with this process there occurs a differentiation of the pars superior into a system of communicating canals and sinuses. These are formed by the outgrowth of a number of pockets and the ingrowth of the walls of a number of deepening grooves. The septa formed by these double-walled epiblastic folds and by the mesoblast included between them result in the formation of a central tri-radiate utriculus, the extremities of which are united by an anterior vertical, a posterior vertical, and a horizontal semicircular canal, each of which possesses a dilated "ampulla," on the floor of which arises a sensory crest or crista acustica. To each of these is distributed a ramus of the auditory nerve. On the medial wall of the utriculus occur a number of sensory epithelial patches, derived, like the epithelium of the cristæ acusticæ, from the neuroepithelium of the pars superior. These macular areas are at first united with one another and with the neuroepithelium of the pars inferior by tracts of neuroepithelium, but in the adult organ they are entirely separated.

The appearance of the grooves and pockets of the pars superior suggests that the anterior vertical and horizontal semicircular canals are formed somewhat earlier than the posterior vertical canal, but, on the other hand, the crista acustica of the horizontal semicircular canal appears to develop somewhat later than do those of the other canals. The development of the canals, the macular areas, and the branches of the auditory nerve has been fully investigated. A well-developed macula neglecta and a nerve ramulus supplied to it were found and the presence of a similar nerve-branch passing to the ductus endolymphaticus was noted. No sensory epithelium was, however, discovered in the saccus endolymphaticus. The pars inferior meanwhile undergoes differentiation into sacculus and cochlea, the latter exhibiting a curved distal pars lagenæ and a proximal pars basilaris, each with its own macular area and nerve supply.

A complete account of the histology of the inner ear was not attempted, but sufficient data have been obtained to confirm and supplement the histological details recorded by Osawa (1898). The maculæ and cristæ acusticæ contain hair-cells (auditory sense epithelium cells) and interstitial (supporting) cells, while the non-sensory areas of the inner ear consist of flattened or cubical epithelium, supported by an external investment of spindle connective tissue.

A detailed investigation of the development of the auditory capsule, columella auris, and associated bones was undertaken, special care being taken to examine a number of embryos in which chondrification of the rudiments of these structures had not yet occurred. The conditions obtaining in such early embryos have now been recorded for—it is believed—the first time, and a full account of the developmental changes undergone by the cartilaginous structures of the auditory region has been given. That the cartilaginous auditory capsule and the anterior cornu of the hyoid are products of two connective-tissue proliferations, and that two centres of chondrification—one hyoidean and one capsular—originally separated by an intervening tract of mesenchyme, are found is indisputable, and, as a result of an examination of the developmental changes undergone by these structures, further information respecting the much-debated question as to the origin and relationships of the columellar apparatus has been obtained.

It is concluded that the columella auris is derived from the hyoid arch, with which it is continuous throughout all stages of development, and that the extra stapedial cartilage is primarily united with the anterior cornu of the hyoid. The supra-stapedial cartilage (including the recurrent process) is developed, and persists as an outgrowth from the extra-stapedial cartilage, and is therefore a hyoidean derivative.

The auditory capsule contributes, at most, a portion of the foot-plate of the stapes, which is probably partly capsular and partly hyoidean in origin. The distal portion of the stapes is exclusively hyoidean. At no period of its life history does Sphenodon possess any cartilaginous attachment between the supra-stapedial cartilage and the cranium, but, during the later stages of the embryonic period, and in adult life, there is a secondary attachment between, and partial fusion of, the supra-stapedial process and the quadrate.

The development of the tympanic cavity, functional tympanic membrane, extra-columellar sinew, and chorda tympani is described. It is noteworthy that, as in the thick embryo, the dorsal portion of the anterior tympanic diverticulum undergoes isolation, and finally disappears.

The results now obtained support the contention advanced by Gray (1913) that, while the inner ear of Sphenodon differs but little from that of other Reptilia—with the exception of Crocodilia—the middle ear really represents a transition stage in the evolution of the middle ear of the living Reptilia.

The Physiological Cost of Muscular Work Measured by the Discharge of Carbon Dioxide. Part II.—*The Energy Output of Labourers on Cold Storage Work.*

By A. D. Waller, M.D., F.R.S., with the assistance of Miss G. de Decker.*

(Received November 22, 1919.)

In pursuance of the investigation of dock labour initiated for the Food (War) Committee of the Royal Society last year, and thanks to the courtesy of several officials of the Port of London Authority and of several willing labourers, we were able to make two series of hourly observations of the CO_2 output of four labourers engaged upon heavy work in cold storage. A first short series was made during December, 1918, at the East Surrey Docks; a second and longer series at the Charterhouse Cold Storage Chambers, Smithfield Market during July, 1919. The data we have been able to obtain are obviously scanty, but, we believe, sufficient to prove the possibility of acquiring valuable information by an expeditious method, applied during normal work, and involving a minimum interference with that work.

Our attempt to submit such scanty results to statistical treatment may perhaps be regarded as premature; we have, however, found it convenient, if only for the purpose of bringing out the necessity of more extensive observations, to work out the standard deviation and the ± probable error in the arithmetic means of the two series of observations made under different conditions.

The first point to arrest attention in cold storage work is the great irregularity in the output of energy as indicated by great fluctuations in the exhalation of CO_2. Cold storage work is piece-work and as such paid for on a higher scale than time-work; it is considered trying work by the labourer, more so in summer than in winter, because of the greater fluctuations of temperature between storage chambers and external air. The work consists principally in the moving from storage van to storage chamber of arrival goods (quarters of beef, of mutton and lamb, boxes of poultry and cheese, etc.), the storage chamber temperature being usually constant at 16° F., *i.e.*, $-9°$ C.

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* The responsibility for this inquiry rests upon two persons, viz., Dr. A. D. Waller, by whom it was initiated and is now described, and Miss G. de Decker, by whom the actual observations were made. The expenses have been met by grants from the Royal Society and from the Medical Research Committee.

Erratum in Part I, p. 167, footnote (first line)—

For Method B *read* Method A.

The principal factor in the fluctuation of energy output is fluctuation of work; the load varies from a very low to a very high level according as small or large consignments come in during the twenty-four hours.

The chief difference we have observed in cold storage work as compared with ordinary work in the open consists in the more marked character of the hourly increased output of CO_2, to which attention was drawn in an earlier portion of this report, and which is best shown by a graph of the day (or night) output. The CO_2 ordinate climbs steeply from hour to hour, from its minimum at the beginning of work.

Our cold storage observations were undertaken with the definite expectation of finding a distinct increase in the physiological cost of work, and we must confess that the correlation that must be believed in has not come up to our expectations. It seems to be masked by the effects of fluctuations of work done. We shall not attempt, therefore, to measure the correlation, and shall simply state results.

Our first set of readings were made for a period of six days, January 30th to February 4th, at the East Surrey Docks; they are given in the subjoined Table and graph.

Cold storage work forms a relatively small proportion of the labour of the East Surrey Docks; the "load" fluctuates accordingly; the men do not like the work, in spite of the fact that it is piece-work and highly remunerated. The energy output of two of the labourers under observation, as indicated by their CO_2 ordinate, measured at hour intervals, was surprisingly low.

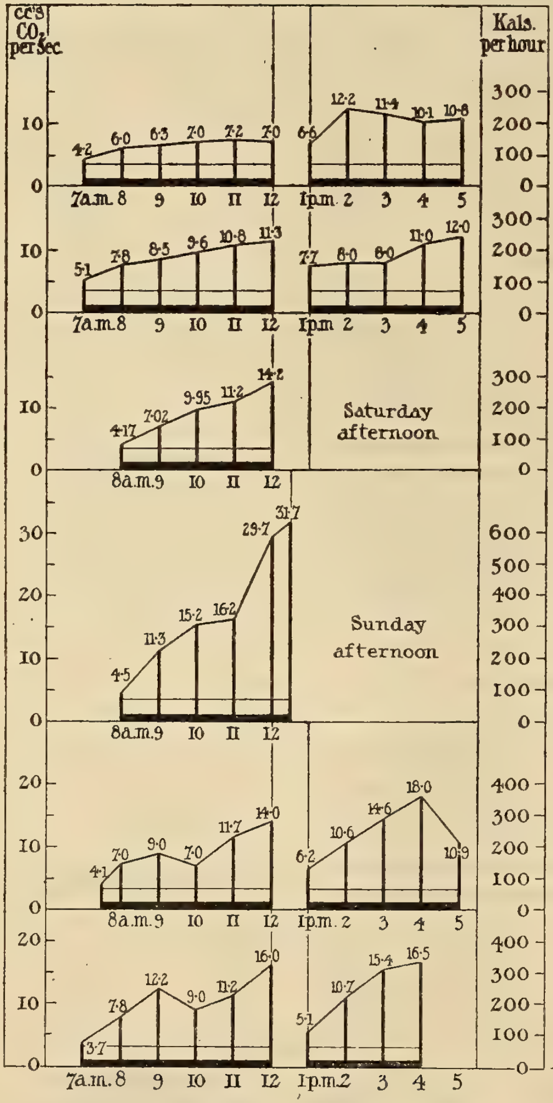
A second set of readings were taken by one of us (G. de D.), by courtesy still of the Port of London Authority, at the cold storage chambers adjacent to Smithfield Market, where the work is more continuous, consisting in the daily (or rather nightly) transfer of meat from cold storage to market, and in more or less irregular bouts of heavy labour occasioned by arrivals of van-loads of meat from ships arriving in harbour. The labourer, standing by or tidying up on his basal wage, is suddenly called upon to work at full pressure on piece-work in a gang where each member is working for the benefit of himself and of his mates. Physiologically, his energy output is now maximal.

These conditions of work afford an excellent opportunity of testing the value of Method B, which serves obviously to give indications of the increasing or diminishing work; but they are not such as to allow of averaging from successive days or nights, as in the case of ordinary dock labour (*v.* Part I). We must evaluate the cost of cold-storage labour from periods of really heavy work, rejecting altogether periods of "standing by," during which the energy output is undoubtedly subnormal. Thus, in the

Labourers Nos. 3, 4, and 5 on Cold Storage Work, East Surrey Docks.

Series A.

Time.	Time of sampling (seconds).	Ventilation (litres).	Ventilation, c.c. per sec.	CO_2 per cent.	CO_2, c.c. per sec.	
7.0	60	9	150	2·8	4·2	Thursday, January 30, 1919. Labourer No. 3.
8.0	60	12	200	3·0	6·0	
9.0	60	11	187	3·4	6·3	
10.0	60	14	234	3·0	7·0	
11.0	60	13	217	3·3	7·2	
12.0	60	12	200	3·5	7·0	
12 to 1 P.M. Dinner hour.						
1.0	60	18	300	2·2	6·6	
2.0	50	18	360	3·4	12·2	
3.0	50	15	300	3·8	11·4	
4.0	60	16	267	3·8	10·1	
5.0	60	18	300	3·6	10·8	
7.0	60	14	234	2·2	5·1	Friday, January 31. Labourer No. 3.
8.0	60	18	300	2·6	7·8	
9.0	60	17	284	3·0	8·5	
10.0	60	18	300	3·2	9·6	
11.0	60	18	300	3·6	10·8	
12.0	60	20	333	3·4	11·3	
12 to 1 P.M. Dinner hour.						
1.0	60	16	267	2·9	7·7	
2.0	60	16	267	3·0	8·0	
3.0	60	15	250	3·2	8·0	
4.0	60	19	317	3·5	11·0	
5.0	60	18	300	4·0	12·0	
7.0	—	—	—	—	—	Saturday, February 1. Labourer No. 3.
8.0	60	10	167	2·5	4·17	
9.0	60	14	234	3·0	7·02	
10.0	60	17	284	3·8	9·95	
11.0	60	16	267	4·2	11·21	
11.45	60	19	316	4·5	14·22	
8.0	60	9	150	3·0	4·50	Sunday, February 2. Labourer No. 4. "Heavy work." "Overtime" pay. Average weight of a forequarter of beef is 1¾ cwt. This labourer moved 22 forequarters, 3 yards, up and down in 1 hour.
9.0	60	20	334	3·4	11·35	
10.0	60	24	400	3·8	15·20	
11.0	60	24	400	4·0	16·20	
12.0	40	26	650	4·5	29·75	
12.30	40	27	675	4·7	31·72	



	Per man.		Per sq. metre per hour.
	Total Kals.	Kals per hour.	Kals (gross) − Kals (basal). = Net Kals cost.
No. 3, Th., Jan. 30.	1490	166	86 − 40 = 46
No. 3, Fri., Jan. 31.	1635	182	94 − 40 = 54
No. 3, Sat., Feb. 1.	747	187	97 − 40 = 57
No. 4, Sun., Feb. 2.	1503	334	197 − 40 = 157
No. 5, Mon., Feb. 3.	1854	218	121 − 40 = 81
No. 5, Tu., Feb. 4.	1739	217	121 − 40 = 81

East Surrey Docks. Labourers Nos. 3, 4 and 5.

Labourers Nos. 3, 4, 5—*continued.*

Time.	Time of sampling (seconds).	Ventilation (litres).	Ventilation, c.c. per sec.	CO_2 per cent.	CO_2, c.c. per sec.	
7.30	60	10	167	2·5	4·17	Monday, February 3. Labourer No. 5.
8.0	60	14	234	3·0	7·02	
9.0	60	18	300	3·0	9·00	
10.0	60	15	250	2·8	7·00	
11.0	60	18	300	3·9	11·70	
12.0	60	20	333	4·2	14·02	
12 to 1 P.M. Dinner hour.						
1.0	60	12·5	208	3·0	6·24	
2.0	60	20	333	3·2	10·60	
3.0	60	22	367	4·0	14·60	
4.0	60	24	400	4·5	18·00	
5.0	60	22	367	3·3	10·90	
7.0	60	9	150	2·5	3·75	Tuesday, February 4. Labourer No. 5.
8.0	60	18	300	2·6	7·80	
9.0	60	23	384	3·2	12·28	
10.0	60	18	300	3·0	9·00	
11.0	60	21	350	3·2	11·20	
12.0	60	24	400	4·0	16·00	
12 to 1 P.M. Dinner hour.						
1.0	60	14	233	2·2	5·14	
2.0	60	10	333	3·2	10·70	
3.0	60	22	367	4·2	15·41	
4.0	60	22	367	4·5	16·50	

case of Labourer No. 7, there was no additional work during the night of Thursday, July 31st, and the CO_2 ordinate was correspondingly low. The average CO_2 (gross) for the last three hours was 6·7 c.c. per second, *i.e.*, allowing 4 c.c. per second for resting CO_2, the working CO_2 was only 2·7 c.c., equivalent to 54 Kals per hour (= 27·96 Kals per hour per metre).

To ascertain the effect of low temperature upon energy output, we must take only periods of piece-work in cold storage, *e.g.*, in the case of Labourer No. 7, the work during the mornings of August 5th, 6th, and 7th was at full pressure, the amount of meat that had to be stored was greater than could be moved during the normal hours, the pay was on the piece-work scale, and the physiological output of energy can be considered as maximal. The figures obtained may, therefore, be compared with those of Labourer No. 2 (given above) for coaling. For these three mornings the average CO_2 ordinate for the last three hours was 24·1, or net 20·1, signifying 402 Kals per hour, or 208·3 Kals per hour per square metre. (King's average for the first three hours of two forenoons of coaling was 20·45 gross, or 16·45 net, signifying 329 Kals per hour, or 168·8 Kals per hour per metre.)

Labourer No. 6 worked continuously at high pressure during the nights of August 5th, 6th, and 7th, and the mean CO_2 for the last three hours of these three nights was 26·53 (gross) or 22·53 (net), signifying 506 Kals per hour or 246·2 Kals per hour per metre.

These are not figures selected to prove any case, but figures collected as a fair random sample under conditions of maximum heavy work in cold storage, fairly comparable with figures afforded by maximum heavy work (coaling) at ordinary temperatures. Their comparison may be facilitated by casting them into tabular form. Obviously they are too few in number to warrant any general conclusion or to be used as a statistical basis.

		No. of observations.	Net CO_2 per sec.	Net Kals per hour per metre.
Labourer No. 2	Coaling	6	16·45	168·8
Labourer No. 6	Cold storage	9	22·53	246·2
Labourer No. 7	,, ,,	9	20·10	208·3

The cases of Labourers Nos. 3 and 5 must be rejected altogether in this connection; they gave low values in cold storage work, but their work, and therefore their energy output, was too intermittent; their CO_2 ordinates or their heat discharge per square metre was below par, much lower than for ordinary piece-work or even time-work.

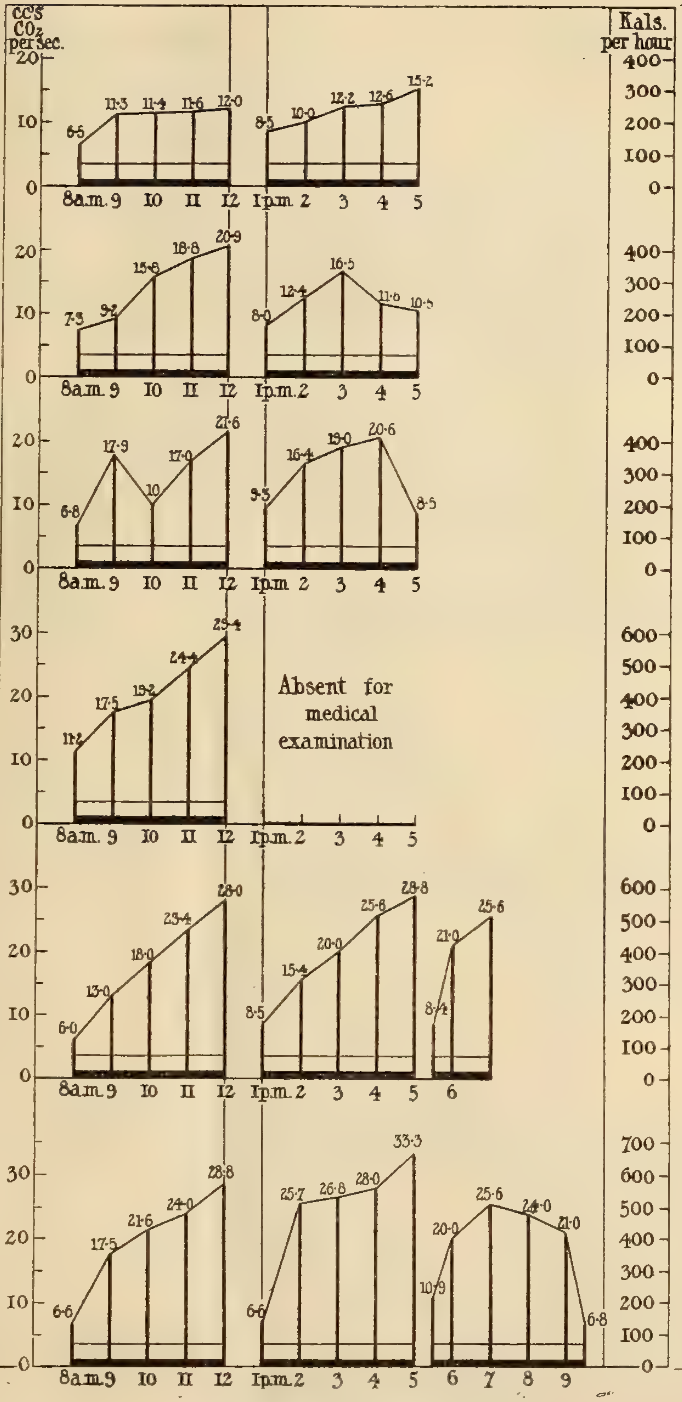
The energy output at the East Surrey Docks during the winter months January and February was, on the whole, much lower than that found at the Smithfield cold storage chambers during the summer months July and August. We do not attribute the difference to the season, but to the difference of labour conditions. The output of energy per man (or per square metre of man-surface) is greater at Smithfield because the daily aggregate of work done was greater.

Labourers Nos. 6 and 7.

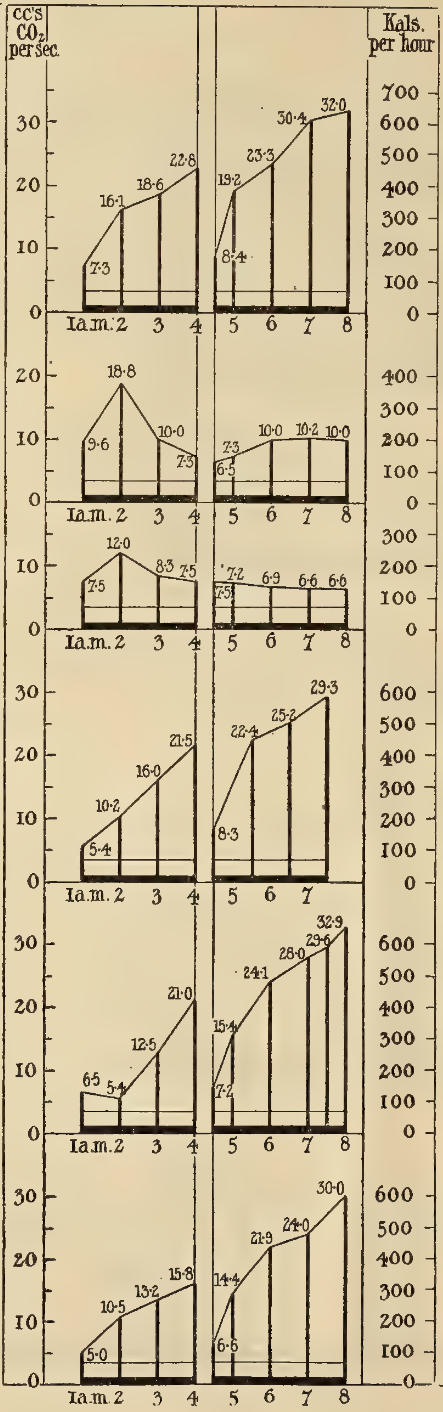
Time.	Time of sampling (seconds).	Ventilation (litres).	Ventilation, c.c. per sec.	CO_2 per cent.	CO_2, c.c. per sec.	
8.0	60	12	216	3·2	6·9	Thursday, July 24, 1919. Labourer No. 6.
8.10	60	13	216	3·0	6·5	
9.0	60	20	333	3·4	11·3	
10.0	60	18	300	3·8	11·4	
11.0	60	20	333	3·5	11·6	
12.0	60	22·5	375	3·2	12·0	
1.0	60	15	250	3·4	8·5	
2.0	60	18	300	3·3	9·9	
3.0	60	21	350	3·5	12·2	
4.0	60	20	333	3·8	12·6	The men called this an easy day.
5.0	60	24	400	3·8	15·2	
8.0	60	13	216	3·4	7·3	Friday, July 25. Labourer No. 6.
9.0	60	15·5	258	3·6	9·3	Working at high pressure from 9 to 12. Six men loaded 1700 carcases of sheep.
10.0	50	22	440	3·6	15·8	
11.0	45	23·5	522	3·6	18·8	
12.0	45	24	550	3·8	20·9	
1.0	60	16	266	3·0	7·9	From 1 to 3, they loaded 240 large quarters of beef.
2.0	45	18	400	3·1	12·4	
3.0	45	22	488	3·4	16·6	From 3 to 5 was a quiet time. No. 6 walked about tidying up and doing odd jobs. No large order.
4.0	60	20	333	3·5	11·6	
5.0	60	18	300	3·5	10·5	
8.0	60	12	200	3·4	6·8	Monday, July 28. Labourer No. 6.
8.10	60	12	200	3·4	6·8	Fairly heavy work to finish off the work of the night men, loading a large consignment of lamb, not cleared during Sunday night.
9.0	35	17	485	3·7	17·9	
10.0	60	20	333	3·0	10·0	
11.0	35	17	485	3·5	16·9	
12.0	35	21	600	3·6	21·6	At 10 A.M., 5 mins. interval for tea.
1.0	60	20	333	2·8	9·3	
2.0	35	16	457	3·6	16·5	
3.0	35	19	542	3·5	18·9	At 5 P.M., found resting, the job had been finished a few minutes before.
4.0	35	19	542	3·8	20·6	
5.0	60	15	250	3·4	8·5	

Labourers Nos. 6 and 7—*continued.*

Time.	Time of sampling (seconds).	Ventilation (litres).	Ventilation, c.c. per sec.	CO_2 per cent.	CO_2, c.c. per sec.	
						Monday, July 28 ("Sunday night"). Labourer No. 7.
12.45	60	17	283	2·6	7·35	A heavy night.
12.55	60	17	283	2·6	7·35	5680 carcases of lamb and sundries to be loaded by 10 men. They actually loaded 4346 carcases and 94 sundries.
2.0	35	18·5	529	3·0	15·87	At 2 A.M., 5 mins. rest for coffee.
3.0	30	17·5	583	3·2	18·65	At 4 A.M., 30 mins. rest for coffee.
4.0	30	19	633	3·6	22·78	
4.30	60	18	300	2·8	8·4	
5.0	30	18	600	3·2	19·2	
6.0	30	20	666	3·5	23·3	
7.0	30	24	800	3·8	30·4	
8.0	30	24	800	4·0	32·0	
						Wednesday, July 30 ("Tuesday night"). Labourer No. 7.
1.0	60	24	400	2·4	9·6	An easy night.
2.0	35	22	628	3·0	18·8	Between 1 and 2 A.M., 10 men loaded 342 carcases of lamb.
3.0	60	20	333	3·0	9·9	At 4 A.M., "No. 7 was dozing and I did not wake him." G. de D.
4.0	—	—	—	—	—	
5.0	60	18	300	2·4	7·2	
6.0	60	24	400	2·5	10·0	
7.0	60	22	366	2·8	10·2	From 5 to 6 he went out. From 6 to 8 he moved about on odd jobs.
8.0	60	20	333	3·0	9·9	
						Thursday, July 31 ("Wednesday night"). Labourer No. 7.
1.0	60	19	316	2·4	7·58	
2.0	60	24	400	3·0	12·0	A very easy night.
3.0	60	20	333	2·5	8·3	"Only six bags of suet to be taken to the market, the rest of the night not working at all."
4.0	60	18	300	2·5	7·5	
5.0	60	18	300	2·4	7·2	
6.0	60	19	316	2·2	6·9	
7.0	60	18	300	2·2	6·6	
8.0	60	18	300	2·2	6·6	



	Total Kals.	Kals per hour.
No. 6, July 24.	1802 124 – 40	225 = 84
No. 6, July 25.	2155 136 – 40	244 = 96
No. 6, July 28.	2478 170 – 40	310 =130
No. 7, Aug. 5.	1628 200 – 40	407 =160
No. 7, Aug. 6.	2634 136 – 40	277 = 96
No. 7, Aug. 7.	5320 218 – 40	443 =178



	Total Kals.	Kals per hour.
No. 7, July 28 (Sunday night).	2719 206 − 40	418 =166
No. 7, July 30.	1391 105 − 40	214 = 65
No. 7, July 31.	1038 78 − 40	159 = 38
No. 6, Aug. 4–5 (Sunday night).	2121 194 − 40	353 =154
No. 6, Aug. 6.	2271 192 − 40	350 =152
No. 6, Aug. 7.	2149 181 − 40	330 =141

Smithfield Cold Storage. Labourers Nos. 6 and 7.

Time.	Time of sampling (seconds).	Ventilation (litres).	Ventilation, c.c. per sec.	CO_2 per cent.	CO_2, c.c. per sec.	
1.0	60	18	300	1·8	5·4	Monday, August 4 ("Sunday night"). Labourer No. 6. "A busy night." Six men had to load 1125 + 200 carcases of lamb and 168 "sundries." They worked very hard all night, except during the half hour interval for coffee from 4 to 4.30 A.M.
2.0	30	11	367	2·8	10·2	
3.0	30	16	533	3·0	16·0	
4.0	30	19	633	3·4	21·5	
4.30	60	20	333	2·5	8·3	
5.30	30	21	700	3·2	22·4	
6.30	30	21	700	3·6	25·2	
7.30	30	22	733	4·0	29·3	
1.0	60	18	300	2·0	6·0	Wednesday, August 6 ("Tuesday night"). Labourer No. 6. Waiting for orders from 1 to 2 in the labourers' sitting room. At 3.30 a heavy order came in; 900 boxes of chickens to be stored by six men. 4 to 4.30 interval for coffee.
2.0	60	18	300	1·8	5·4	
3.0	30	15	500	2·5	12·5	
4.0	30	21	700	3·0	21·0	
4.30	60	18	300	2·4	7·2	Heavy work.
5.0	30	16	533	3·0	16·0	
6.0	30	22	733	3·3	24·2	
7.0	30	24	800	3·5	28·0	
7.30	30	24	800	3·7	29·6	
7.55	30	26	867	3·8	32·9	
1.0	60	17	283	1·8	5·1	Thursday, August 7 ("Wednesday night"). Labourer No. 6. "A busy night." 1867 + 75 carcases of lamb to be carried by six men. Heavy work all night, except from 4 to 4.30 for coffee.
2.0	30	15	500	2·1	10·5	
3.0	30	18	600	2·2	13·2	
4.0	30	17	567	2·8	15·8	
4.30	60	20	333	2·0	6·7	
5.0	30	18	600	2·4	14·4	
6.0	30	22	733	3·0	22·0	
7.0	30	24	800	3·0	24·0	
7.50	30	26	867	3·5	30·0	
8.0 }	60	25·0	416	2·8	11·6	Tuesday, August 5. Labourer No. 7. *Note by* G. de D.—After the first reading showing an unusually high CO_2 value, I made him rest in my office for 6 mins., and took a second reading which was practically the same. I asked him if he had had a good night, and he replied that he had been worried about the possible result of a medical examination, because he had been classified as B. 3 while in the Army. To all appearance he is A 1.
8.6 }	60	24·0	400	2·8	11·2	
9.0	30	17·0	567	3·1	17·5	
10.0	30	18·0	600	3·2	19·2	
11.0	30	24·5	816	3·0	24·5	
12.0	30	26·0	867	3·4	29·4	
Absent for medical examination for promotion as "regular" labourer.						

Labourers Nos. 6 and 7—*continued.*

Time.	Time of sampling (seconds).	Ventilation (litres).	Ventilation, c.c. per sec.	CO_2 per cent.	CO_2, c.c. per sec.	
8.0	60	18	300	2·0	6·0	Wednesday, August 6. Labourer No. 7. "There are 6000 cases of chickens to be stored and the men will be busy all day long."
9.0	30	15	500	2·6	13·0	
10.0	30	18	600	3·0	18·0	
11.0	30	22	733	3·2	23·4	
12.0	30	24	800	3·5	28·0	
12 to 1 P.M. Dinner hour.						
1.0	60	15	250	3·4	8·5	
2.0	30	21	700	2·2	15·4	
3.0	30	24	800	2·5	20·0	
4.0	30	24	800	3·2	25·6	
5.0	30	24	800	3·6	28·8	
5.30	60	21	350	2·4	8·4	
6.0	30	21	700	3·0	21·0	
6.30	30	24	800	3·2	25·6	
8.0	60	20	333	2·0	6·7	Thursday, August 7. Labourer No. 7. A very heavy day. Six men loaded 855 sheep, 2773 lambs, and 3230 boxes of chickens. (The day's pay was £1 4*s.* 8½*d.* per man.)
9.0	30	21	700	2·5	17·5	
10.0	30	24	800	2·7	21·6	
11.0	30	24	800	3·0	24·0	
12.0	25	24	960	3·0	28·8	
12 to 1 P.M. Dinner hour.						
1.0	60	18	300	2·2	6·7	
2.0	25	23	920	2·8	25·8	
3.0	30	26	866	3·1	26·8	
4.0	30	24	800	3·5	28·0	
5.0	30	25	833	4·0	33·3	Rest for tea from 5 to 5.30.
5.30	60	22	366	3·0	11·0	
6.0	30	20	666	3·0	20·0	Feels "very tired," but goes on working at full pressure.
7.0	30	24	800	3·2	25·6	
8.0	30	24	800	3·0	24·0	N.B. — Diminishing CO_2 which indicates that he is overworked and that his output is falling.
9.0	30	21	700	3·0	21·0	
9.30	30	18	600	2·8	16·8	

ADDENDUM 1.—*On a Numerical Value for "Efficiency."*

The calibration of a labourer is essentially completed when his rate of CO_2 discharge has been determined.

A further step may be taken by measuring the *efficiency* of a given labourer for a given piece of artificial work of which the mechanical value is known—

in foot-pounds per minute or horse-power,* of, preferably, in kgrm.-metres per second.

I take as the measure of physiological efficiency the ratio

kgrm.-metres per second/cubic centimetres CO_2 per second,

i.e., the number of kgrm.-metres per 1 c.c. CO_2 [or by its reciprocal CO_2/kgrm.-metre, *i.e.*, the physiological cost in CO_2 of 1 kgrm.-metre of work].

The expression kgrm-metre/CO_2 is further simplified by conversion of the CO_2 value into its equivalent mechanical value in kgrm.-metres. For this conversion I take the factor 2·5, which gives a single numerical value of physiological efficiency. Or the value of the kgrm.-metre/CO_2 ratio multiplied by 40 gives at once in per 100 the "efficiency" value of the subject.

For example, a given subject (A. D. W.), working for two minutes at the net cost of 25 c.c. CO_2 per second has a kgrm.-metre/CO_2 ratio = 20/25 = 0·8, and an efficiency = 0·8 × 40 = 32 per cent. His CO_2/kgrm.-metre ratio is 25/20, or 1 kgrm.-metre of his work (at the rate given) costs 1·25 c.c. of CO_2

On this principle, a further calibration of Labourer No. 1 is subjoined: 1st, by staircase work, and 2ndly, by walking on a horizontal path.

September 6th, 1919.—Labourer No. 1. Weight, 87·2 kgrm.; staircase ergometry, three ascents of a 20-metre staircase; samples of expired air collected during the last 5 metres of each ascent, *i.e.*, during 20 to 21 secs.; descents by lift in 30 to 40 sec.

	Time of sample (seconds).	Ventilation.		CO_2 per cent.	CO_2, c.c. per sec.
		Litres.	c.c. per sec.		
First trial—					
Normal at rest	60	8	133	3·0	4·0
1st ascent	18	11	611	4·0	24·4
2nd ,,	21	20	952	3·8	36·2
3rd ,,	20	20	1000	3·6	36·0

$$\text{Work per sec.} = \frac{87 \cdot 2 \times 20}{82} = 21 \cdot 27 \text{ Kg.-M.}$$

Cost of work per sec. = 36·1 − 4 = 32·1 cc. CO_2.

$$\frac{\text{Kg.-M.}}{CO_2} = \frac{2127}{3210} = 0 \cdot 66 \times 40 = 26 \cdot 4 \text{ per cent. efficiency.}$$

CO_2/Kg.-M. = 1·515 (= 8·4 Kals).

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* 1 horse-power = 550 foot-pounds per minute, or approximately 75 kgrm.-metres per second.

September 6th, 1919.—Labourer No. 1—*continued.*

	Time of sample (seconds).	Ventilation.		CO_2 per cent.	CO_2, c.c. per sec.
		Litres.	c.c. per sec.		
Second trial (after ½ hour of rest)—					
1st ascent	20	15·5	775	3·8	29·45
2nd "	21	20·4	971	3·8	36·90
3rd "	21	23·0	1095	3·4	37·23

Work per sec. $= \frac{87 \cdot 2 \times 20}{84} = 20 \cdot 76$ Kg.-M.

Cost of work per sec. $= 37 \cdot 06 - 4 = 33 \cdot 06$ c.c. CO_2.

$\frac{\text{Kg.-M.}}{CO_2} = \frac{2076}{3306} = 0 \cdot 63 \times 40 = 25 \cdot 2$ per cent. efficiency.

CO_2/Kg.-M. $= 1 \cdot 587$ c.c. ($= 8 \cdot 8$ calories).

Note.—The lower efficiency in the second as compared with the first trial is perhaps due to fatigue. The subject said at the end of the second trial that it was very hard work, much harder than the garden trial.

Labourer No. 1. Walking at 3·8 miles per hour on a horizontal path; 15 laps at 216 metres per lap in 30 min.; speed 2 min. per lap or 1·8 metres per second; samples of expired air taken for about ½ min. for the first quarter of each of the first three laps and thereafter at the 6th, 9th, 12th, and 15th lap. The readings were as follows:—

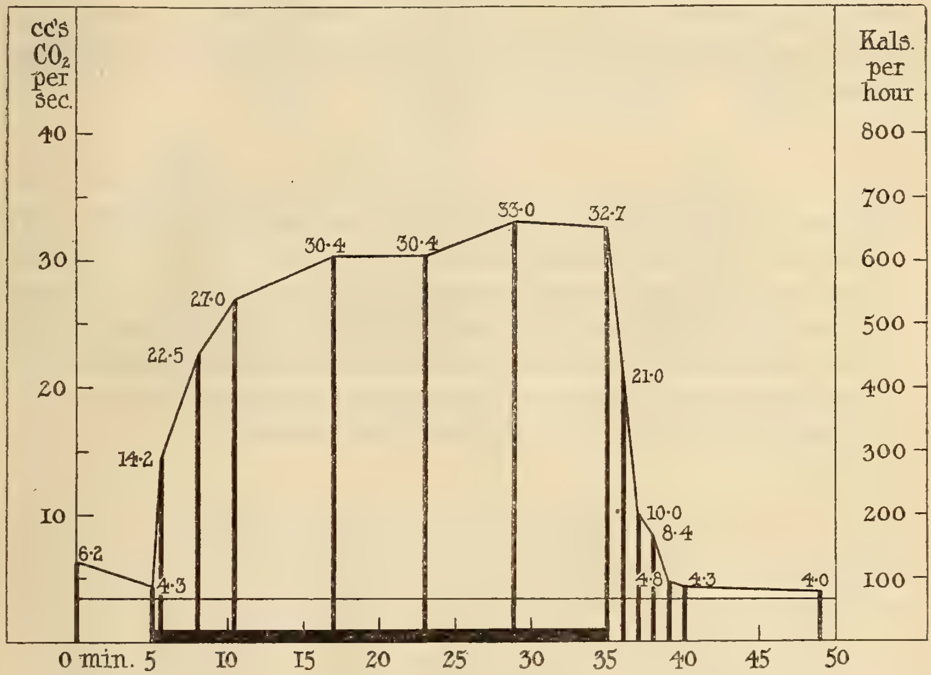
	Time of sample (seconds).	Ventilation.		CO_2 per cent.	CO_2, c.c. per sec.	
		Litres.	c.c. per sec.			
—	60	12·5	—	3·8	6·2	
At rest	60	10	167	2·6	4·34	
1st lap	32	13	406	3·5	14·21	
2nd "	32	19	594	3·8	22·57	
3rd "	28	18	643	4·2	27·00	
6th "	26	18	692	4·4	30·45	Average 31·66
9th "	25	19	760	4·0	30·40	
12th "	23	20	870	3·8	33·06	
15th "	22	20	909	3·6	32·72	
At rest, 1st min.	30	18	600	3·5	21·00	
2nd min.	30	10	333	3·0	10·00	
3rd "	50	15	300	2·8	8·40	
4th "	50	10	200	2·4	4·80	
5th "	50	9	180	2·4	4·32	
10th "	60	10	167	2·4	4·01	

$\frac{\text{Kg.-M. (hor.)}}{CO_2} = \frac{156 \cdot 96}{27 \cdot 66} = 5 \cdot 676.$

CO_2/Kg.-M. (hor.) $= 0 \cdot 1762$.

1 kgrm.-metre (hor.) costs 0·1762 c.c. CO_2 (= 0·98 calorie),
1 " proper " 1·587 " (= 8·88 calories),
∴ 1 kgrm.-metre proper = 9·01 kgrm.-metre (hor.),

or, in words, the cost of raising the body 1 metre (at a speed of 0·25 metre per second) will carry the body horizontally 9·01 metres at a speed of 1·8 metres per second.



Labourer No. 1.—Walking at 1·8 metres per second. Weight = 87·2 kilom.

The "20-metre staircase" in connection with the Physiological Laboratory of the University of London, used for estimating work in these observations, includes an allowance for the horizontal path involved in walking upstairs. The staircase has 103 steps, 7-inch rise, 9-inch tread, and short landings, making up a total horizontal distance of 28 yards, of which the vertical value has been assumed at one-fifteenth. The effective value of the 20 yards is thus 20 + 1·867 yards, *i.e.*, 20 metres. The value of the horizontal in terms of vertical was assumed as one-fifteenth in accordance with the findings of Zuntz and others, but from my own observations (unfinished) I am disposed to take a larger fraction, *i.e.*, about one-tenth, for the relation for moderate speeds, viz., 100 yards per minute, on the flat, or 10 yards per minute vertically. The point will be more fully considered in a future report on the energy output of walking and running on the flat. If the fraction one-tenth should prove nearer the mark than one-fifteenth, a corresponding correction of the horizontal allowance will have to be made for the 20-metre staircase;

it would then be 20·8 metres, and its work values as now calculated would have to be raised by 4 per cent.

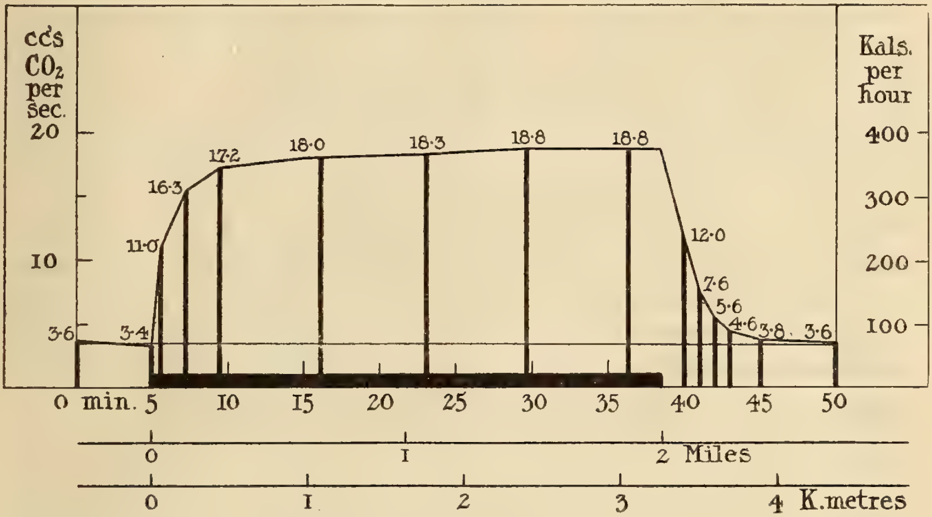
Ordinary walking at moderate speeds, between 3 and 4 miles per hour, is an exercise common to workers of all classes, "sedentary" and "heavy," that affords a very convenient term of comparison between different persons, and with due precautions that can be conveniently utilised for their calibration in terms of CO_2.

A. D. W. Age 63; weight, 82 kgrm.; height, 172 cm. (surface, 1·94 sq. metres). Horizontal walk, 15 times round garden (216 metres per lap), *i.e.*, 3240 metres (approximately two miles) in 33 min. 45 sec., *i.e.*, at a speed of 1·6 metres per second, or 3½ (3·58) miles per hour. Samples of expired air collected at the beginning of each lap for 35 sec. for the first three laps and subsequently every third lap; measured and analysed at once for CO_2. At the end of the walk, 50 sec. samples taken at each minute for 4 min., then at 2 min. and 5 min. intervals.

Lap.	Time.		Time of sample (seconds).	Litres.	c.c. per sec.	CO_2 per cent.	CO_2, c.c. per sec.
—	0′ 00″	At rest	100	12·0	120	3·0	3·6
—	5 00	"	100	11·5	115	3·0	3·4
1	—	Start	35	11·0	314	3·5	11·0
2	7 15		35	15·0	429	3·8	16·3
3	9 30		35	15·0	429	4·0	17·2
6	16 15		35	15·0	429	4·2	18·0
9	23 00		35	16·0	457	4·0	18·3
12	29 45		35	15·0	429	4·4	18·8
15	36 30		35	15·0	429	4·4	18·8
	38 45	Finish.					
	40		50	15·0	300	4·0	12·0
	41		50	10·0	200	3·8	7·6
	42		50	8·0	160	3·5	5·6
	43		50	7·0	140	3·3	4·6
	45		50	6·0	120	3·2	3·8
	50		50	6·0	120	3·0	3·6

Inspection of the graph shows that the gross expenditure of CO_2 during the walk was slightly above 18 c.c. per second, the resting CO_2 before and after the walk about 3·5 c.c.; the net cost of the walk was therefore at the rate of 14·5 c.c. per second, or 290 Kals per hour. The body surface being 1·90 sq. metres, the cost was 152 Kals per square metre. The net cost per horizontal kgrm.-metre comes out as 0·6 calorie approximately.

On inspection of this graph, it will be seen that the "resting CO_2" = 3·6 c.c per second, and the working CO_2 approximately 18 c.c. (gross); or $18-3{\cdot}6 = 14{\cdot}4$ net.



This graph shows further, that the change from resting CO_2 (3·6) to working CO_2 (18·0) at the outset of work and the return at the end of work are complete in about 10 min. Practically the change may be considered as effected in about 5 min.

The value (gross) of working CO_2 is given by any one of the last four ordinates, or better by their arithmetic mean, 18·475.

The gross cost of work = 18·475
The resting CO_2 = 3·6

The net cost of work = 14·875

i.e., walking at 3½ miles per hour costs A. D. W. nearly 15 c.c. CO_2 per second, which, if kept up for 8 hours, must be reckoned as hard work—approximately 2,400 Kals—at 300 Kals per hour.

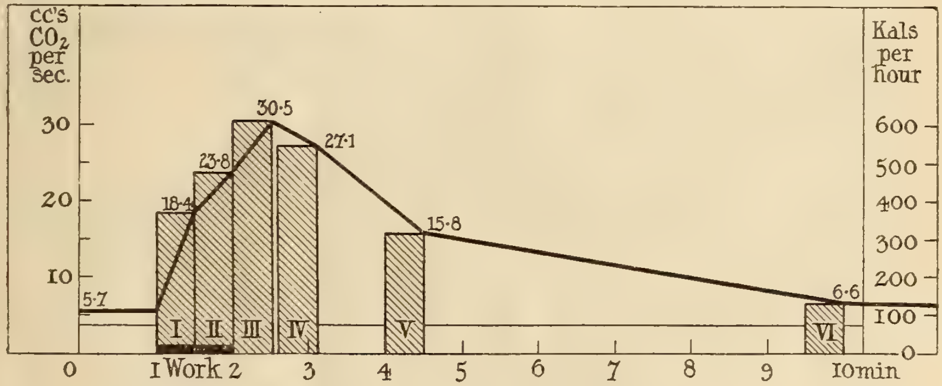
The superficial resemblance of this graph to a record of muscular tetanus will be obvious to any physiologist; the record of a wave of CO_2 passing out of the body in consequence of a brief effort will be similarly recognised. Objection may be raised to my interpretation of this second record to the effect that by reason of the increased pump action—by increased pulmonary suction—short samples cannot be trusted as indicating alterations of CO_2 production. With the involuntary deepening of respiration occurring during and after a brief spell of muscular effort, there is no doubt increased pumping, but there is also increased CO_2 production in the background; both effects are the physiological sequelæ of the muscular effort, and the total resultant CO_2 expired is to be regarded as being in measure with the effort that has produced increased breathing. I possess a large number of data, extending back over many years, by which I have sought to ascertain the quantitative relations between work and cost of work—work measured in kgrm.-metres per second, cost of work measured in cubic centimetres per second and translated into the mechanically equivalent kgrm.-metres—from which I may quote an observation and graph on myself, which is given to serve as a picture showing the kind of time-relations of a single wave of CO_2 exhaled by the lungs in consequence of a short, sharp muscular effort, *i.e.*, the ascent of a 20-metre staircase in one minute, *i.e.*, approximately 80 × 20 kgrm.-metres per minute, or 26·7 kgrm.-metres per second, or 0·33 horse-power. These are rounded figures; actual figures observed are given in the protocol.

The factor × 20 for the conversion of cubic centimetres CO_2 per second to Kals per hour is taken as inclusive of an average temperature correction (15°) for expired air at an average respiratory quotient (0·85). At 0·85 respiratory quotient the factor is 21·081, *i.e.*, 5·4 per cent. above 20. At 15° the volume of CO_2 is 5·5 per cent. above that at 0°. So that the factor 20 practically eliminates the two differences, and the result in Kals per hour is substantially correct.

The value of resting CO_2 is, whenever possible, to be actually measured and used as a base-line from which to measure net CO_2. Practically I take as base-line the CO_2 reading of the subject after sitting at rest for 5, or preferably, 10 minutes. When this is not possible, I assume as base line the value 2 c.c. CO_2 per second per square metre, *i.e.*, 40 Kals per hour per square metre. This value, although perhaps a trifle high, does not sensibly impair the correctness of heavy work values = 20 or 30 c.c. per second, but it is obviously inadmissible for use when the net cost of light work = 5 to 10 c.c. per second is under examination. In the latter case, at least two direct measurements of resting CO_2 at 5 minutes' intervals must be taken before work is begun and after work has ended.

February 6. A. D. W. Weight = 83 kgrm. Exhalation of CO_2 in consequence of a brief period of work (ascent of a 20-metre staircase in 62 seconds). The periods during which samples were collected are shaded.

Time.	Conditions.	Time of sample, secs.	Ventilation.		CO_2.	
			Litres.	c.c. per sec.	per cent.	c.c. per sec.
h. m. s.						
2 40 0	Ordinary occupations in the laboratory	100	14·2	142	3·4	4·83
0 57 0		100	16·6	166	3·7	6·14
3 16 0		100	16·0	160	3·5	5·60
4 0 0		100	15·4	154	3·7	5·70
4 1 31	First 10 metres ascent	31	13·6	439	4·2	18·44
4 2 3	Second 10 metres	32	14·4	450	5·3	23·85
4 2 40	Sitting	30	18·3	611	5·0	30·55
4 3 10	Walking	30	16·6	554	4·9	27·14
4 4 30	Ordinary occupations	50	18·8	376	4·2	15·79
4 10 0		50	9·5	190	3·5	6·65
4 20 0		100	16·0	160	3·7	5·92



Addendum.—April 7, 1920.

The following table is subjoined in order to facilitate reference and comparison with other data, to be given, it is hoped, in further communications:—

Labourer.	Age.	Weight.	Height.	Surface.
No. 1	51 years	80 kilos.	1·69 M.	1·90 M².
„ 2	58 „	85 „	1·73 „	2·00 „
„ 3	55 „	73 „	1·81 „	1·93 „
„ 4	54 „	61 „	1·68 „	1·69 „
„ 5	47 „	68 „	1·73 „	1·80 „
„ 6	32 „	73 „	1·68 „	1·82 „
„ 7	34 „	75 „	1·91 „	2·03 „

Labourer.	—	Total Kals of the day's work.	Hours of work.	Kals gross per hour.	Kals gross per M^2 per hour.	Rest value.	Kals net per M^2 per hour.	—
No. 1	Surrey Docks—Day work on time scale of pay	1976	7	**282**	148	−40	=**108**	
		2298	7¼	**317**	164	40	**124**	
		2262	7½	**302**	159	40	**119**	Aver. = 117±2·6
No. 2	Surrey Docks—On piece-work	2843	7	**406**	203	40	**163**	
	Mixed work ...	2312	6	**385**	192	40	**152**	
	Piece-work ...	2708	6¾	**401**	200	40	**160**	
	On time work	2010	7	**287**	143	40	**103**	
	Do.	1790	6½	**286**	143	40	**103**	Aver. = 128±8·7
	Mixed work ...	1709	6¾	**253**	126	40	**86**	
No. 3	Surrey Docks—Cold storage	1490	9	**166**	86	40	**46**	
		1635	9	**182**	94	40	**54**	
		747	4	**187**	96	40	**56**	
No. 4	—	1503	4½	**334**	186	40	**146**	
No. 5	—	1854	—	**218**	121	40	**81**	Aver. = 77±9·2
		1739	—	**217**	120	40	**80**	
No. 6	Smithfield—Cold storage, day work	1802	8	**225**	123	40	**83**	
		2155	8	**244**	134	40	**94**	
		2478	8	**310**	170	40	**130**	
No. 7	—	1628	4	**407**	200	40	**160**	
		2634	9½	**277**	136	40	**96**	Aver. = 123±9·8
		5320	12	**443**	218	40	**178**	
No. 6	Smithfield—Cold storage, night work	2121	6	**353**	193	40	**153**	
		2271	6½	**350**	192	40	**152**	
		2149	6½	**330**	178	40	**138**	
No. 7	—	2719	6½	**418**	206	40	**166**	
		1391	6½	**214**	105	40	**65**	Aver. = 119±10·7
		1038	6½	**159**	78	40	**38**	

Experimental Researches on Vegetable Assimilation and Respiration. XIII.*—*The Development of Photosynthetic Activity during Germination.*

By G. E. Briggs, Demonstrator in Plant Physiology in the University of Cambridge.

(Communicated by F. F. Blackman, F.R.S. Received March 29, 1920.)

Introduction.

We must assume that the development of every leaf begins with a stage in which it is incapable of performing the function of assimilation. It is a matter for investigation to determine whether this stage is passed when the tiny leaf first becomes visible in the bud or whether it persists even after the leaf is unfolded and fully green.

For seedlings this matter is very important biologically, because it determines when the plant can begin to make additions to its dry weight and start its natural development. Analysis of results of growth experiments on such plants as maize indicates that the early assimilatory power is very feeble.†

In most methods of experimenting on the relation of photosynthetic activity to the development of chlorophyll-greenness the work is complicated by the fact that during the period of illumination of the experiment the chlorophyll continues to increase in amount so that one cannot maintain a constant greenness for precise investigation. A method of experimentation devised by Dr. F. F. Blackman, and used in this laboratory, allows photosynthesis to be measured in an atmosphere with so low an oxygen pressure that further chlorophyll development is inhibited, and the green tone can be kept constant for days while the leaf is exposed to light.

With this procedure the development of photosynthesis has been followed from about zero to nearly full activity. The results, besides throwing light on the nature of the photosynthetic mechanism, explain why Willstätter failed to confirm the results obtained by Irving* in this laboratory in 1910.

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* Since the publication of Part IX ('Roy. Soc. Proc.,' B, vol. 83, p. 389) of this series, carried out in the Botany School, Cambridge, under the general direction of Dr. F. F. Blackman, the following parts have appeared elsewhere:—X. A. A. Irving, "The Effect of Chloroform upon Respiration and Assimilation," 'Annals of Botany,' vol. 25, p. 1077 (1911). XI. D. Thoday, "On the Effect of Chloroform on the Respiratory Exchanges of Leaves," 'Annals of Botany,' vol. 27, p. 697 (1913). XII. A. M. Smith, "The Temperature-Coefficient of Photosynthesis," 'Annals of Botany,' vol. 33, p. 517 (1919).

† Briggs, G. E., Kidd, F., and West, C., "Quantitative Analysis of Plant Growth," 'Ann. Appl. Biol.,' vol. 7, No. 1 (1920).

At this point I wish to express my thanks to Dr. F. F. Blackman for suggesting the research and for advice and criticism.

SECTION I.—*The Relation of Photosynthetic Activity to Age and Chlorophyll Development in Young Leaves.*

Method and Procedure.—The principle of Dr. Blackman's new method for measuring the output of oxygen during photosynthesis is to illuminate the leaf in a small closed chamber in an atmosphere of hydrogen with added carbon dioxide. The oxygen produced is determined by circulating the atmosphere over palladium black, when the oxygen unites with twice its own volume of hydrogen. The resulting reduction in gas volume is measured by means of a gas burette in connection with the apparatus. As this reduction is threefold that of the oxygen produced, very small amounts of assimilation can be accurately measured. The volume of the synthetic water is of course negligible.

The illumination was supplied by an ordinary incandescent filament lamp of thirty-two candle power, and was varied for certain test experiments by changing the distance of the lamp from the chamber.

The percentage of carbon dioxide in the apparatus was about five, and was never allowed to become so small as to limit the rate of photosynthesis.

Various materials, including the young buds of the bean (*Vicia Faba*), the young leaves of the oat (*Avena sativa*), and of the dwarf French bean (*Phaseolus vulgaris*), were used in the preliminary experiments, but the last-mentioned was found to be the most suitable, and was used for the more critical experiments; in *Phaseolus* the leaf-area did not change during the experiments, whilst in the case of the others, slight unfolding of the leaves took place.

Prior to sowing, the seeds were soaked for a short time in a small quantity of water at the temperature of the laboratory (12°–15° C.). When the seeds were swollen they were sown in soil in pots and germinated in a hot box (24°–25° C.) protected from the action of light with a black cloth. For the experiments young yellow leaves were cut from these etiolated plants and exposed to light until they had acquired the desired tone of greenness. The light was usually that of an incandescent filament lamp in a dark room, and was of moderate intensity, so that no retardation of greening was caused by too strong or too weak an illumination. The leaves, when sufficiently greened, were transferred to the apparatus, and their photosynthetic activity measured by their output of oxygen. Except where otherwise stated, they were transferred to the apparatus immediately after exposure to light. Between the periods during which the assimilation was measured, the chamber was disconnected from the apparatus, the hydrogen replaced by air, and the chamber, with the leaves still in it, placed in the dark. In this way the leaf was kept at one tone of greenness for many days.

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* Irving, A. A., "The Beginning of Photosynthesis and the Development of Chlorophyll," 'Annals of Botany,' vol. 24, pp. 805–818 (1910).

At the completion of the experiment each set of leaves was dried at 100° C. until no further loss of weight was observed.

Results.—The details of the experiments on *Phaseolus* set out in the Appendix are summarised in Table A below.

The assimilatory power is expressed as the output of oxygen in cubic centimetres per gramme dry-weight of leaf per hour. The volume is reduced to 0° C. and 760 mm. of mercury. It is realised that this is a measure of "apparent" and not of "real" assimilation, since oxygen consumption, due to respiration, is neglected, and consequently, when the assimilatory power is given as x c.cs., a value of about 1·3 c.cs., as shown by the respiration experiments, should be added. The "apparent" assimilation, however, is suitable for the comparative purposes of these experiments, and to substantiate the conclusions drawn.

Table A.—*Phaseolus vulgaris.* Assimilatory Power of Young Seedling Leaves of different Ages and Degrees of Greenness as Measured by Oxygen Production.

Day from sowing.	Assimilatory power.				
	Expt. I.	Expt. IA.	Expt. II.	Expt. IIA.	Expt. VI.
0–7th	Grown in dark.				
8th	Greened by lighting in air.				
9th	1·3	In dark.			
10th	2·5	4·1			
11th	In dark	5·5	—	—	Greened by lighting in air.
12th	7·0	6·7	—	—	,, ,,
13th	7·0	—	Greened by lighting in air.		,, ,,
14th	—	—	7·8	In dark	,, ,,
15th	—	—	9·5	,,	,, ,,
16th	—	—	10·0	10·5	,, ,,
17th	—	—	—	—	12·0

Table A—*continued.*

Day from sowing.	Assimilatory power.				
	Expt. III.	Expt. IIIA.	Expt. IV.	Expt. IVA.	Expt. V.
0–7th	Grown in dark.			Grown in dark.	
8th	Greened by lighting in air.				
9th	0	In dark.			
10th	5·0	”			
11th	10·4	”			
12th	—	9·2	Greened by lighting in air.		
13th	—	—	6·6	In dark.	
14th	—	—	7·8	”	
15th	—	—	9·1	7·3	
16th	—	—	—	7·0	Greened on plant.
17th	—	—	—	—	” ”
18th	—	—	—	—	” ”
19th	—	—	—	—	” ”
20th	—	—	—	—	12·0

NOTE.—In Expts. I, IA, II, IIA, IV, and IVA, the period of greening was approximately the same, and likewise the tone of greenness ("green-yellow") developed.

The leaves of Expts. III and IIIA were greened for a longer period, and the tone of green obtained was deeper ("yellow-green"). In Expts. V and VI the leaves were full normal green and are included for comparative purposes.

The conditions during illumination were the same in all experiments. Temperature 11°–16° C.

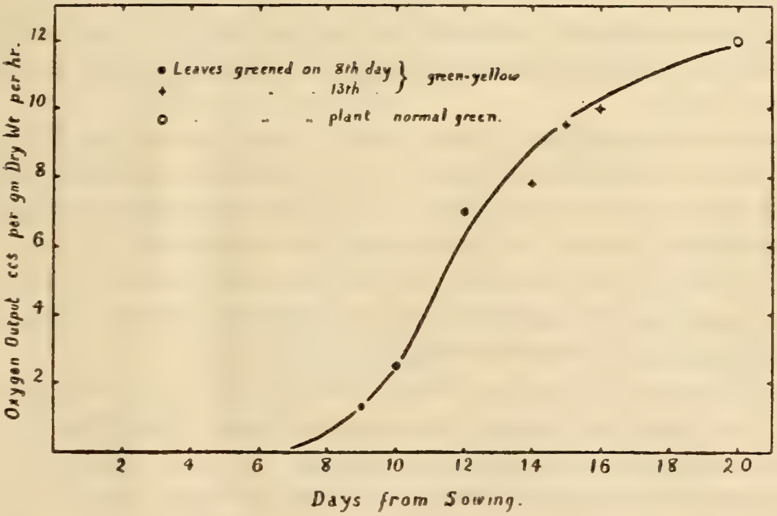
The value given for the assimilatory power is the average for the period during which the leaves were illuminated in the apparatus—a preliminary of about one hour being allowed in each case. For details see Appendix.

The first noticeable feature of the results recorded is that the assimilatory power of leaves, which have developed a very small portion of their normal content of green pigment, is a quantity which depends more upon the age of the leaf than upon its colour. The leaves of Expts. I and II are comparable in that they are from plants which were sown together. The main difference is that the leaves of Expt. I were exposed to light on the eighth day from sowing, whilst those of Expt. II were five days older when exposed. The assimilation of the former on the first day after greening was only 1·3 units, whilst that of the older leaves was 7·8. Still more striking is a comparison of Expts. III and IV. The younger leaves of Expt. III produced no surplus oxygen during the seven hours they were in the apparatus. These leaves had been exposed to light for 25 hours, and during that time had developed a yellow-green colour. On the other hand, those of Expt. IV, which were four days older, showed an output of 6·6 units of oxygen, although they had been exposed to light only 13 hours, during which time they had become only green-yellow.

The second feature of note is that, whatever the amount of the oxygen output on the first day after greening, it increased during the following days until it reached a value, in some cases, almost as large as that of leaves which had been exposed for three or more days, and had attained a normal green colour. Reference to Expt. III will reveal that leaves, which on the first day showed no assimilation, on the third day had reached a value of 10·4, whilst the normal leaves of Expts. V and VI under similar conditions gave a value of 12 units. Yet throughout each increasing series there was no increase in greenness of the material.

Moreover, if the leaves after greening are kept in the dark for three days, they show at the end of that period an assimilatory power of the same dimensions as that of similar leaves, which have been exposed to light in the apparatus during a considerable part of the three days. For example, the leaves of Expt. IIIA received the same treatment as those of Expt. III, except that they were in the dark whilst the latter were in the apparatus. At the end of three days, however, they showed an assimilation of 9·2 units as compared with the 10·4 of Expt. III—values almost the same.

That the above features are general for such partially greened etiolated leaves is borne out by numerous results obtained with other material. Additional evidence from *Avena* and *Vicia* is set out in the Appendix.



The results of Expts. I and II, comparable in that the leaves were of similar tones of greenness, are presented graphically in the accompanying figure, where the abscissæ are days counted from sowing and the ordinates oxygen output in c.cs. per grm. dry-weight per hour. The value for the fully green leaves of Expt. VI is included for comparative purposes.

Seeking the interpretation of these phenomena we may dispose of certain factors to which the increase of assimilation, with progress of time after greening, might have been attributed in the first instance. These are (1) increase in chlorophyll-content during that time; (2) increase in leaf area; (3) fall in respiration, since the assimilation measured is the "apparent" not the "real" assimilation; or, possibly (4) a combination of these factors.

It seems most improbable that the increase is due to increased chlorophyll-content, since the same increase in assimilation was observed when the leaves were kept in the dark. To gain definite information on this point one sample of leaves was taken directly after greening, and another sample of the same lot after exposure to light in the apparatus; both samples were dried and extracted with 85 per cent. acetone, in such quantities that the ratio of dried leaf material to extract was the same in both cases. These extracts were indistinguishable in tone of greenness, thus showing that the conditions of the experiment were really such as to inhibit further development of chlorophyll.

The change in size of the leaves during the experiments was found to be negligible.

Knowing that the respiration of young leaves is relatively large, it was decided to determine its magnitude for material similar to that used in the assimilation experiments, and to measure the falling respiration during a three-day period.

To estimate the respiration, about twenty young etiolated leaves of six or seven days from sowing were placed in an air-tight flask containing ordinary air; the flask was placed in the dark, and at intervals samples of gas were withdrawn and analysed for carbon dioxide, and at the same time the gas in the flask was replaced by ordinary air. The results are recorded in Table XII in the Appendix, the respiration being expressed as cubic centimetres of carbon dioxide per gramme dry weight of leaf per hour.

The first value, 1·4, is most probably too high, as it represents the rate of respiration during the five hours subsequent to cutting: respiration is usually greatly increased by wounding and handling. If it be assumed that the respiration of these leaves decreased from 1·3 to 0·8 units during the three days, then the magnitude of the fall in respiration is not likely to be under-estimated. Consequently the fall in respiration cannot be held to account for a rise in "apparent" assimilation of more than 0·5 units, unless the coefficients of gaseous exchange depart very widely from unity. Moreover, this fall in respiration is due chiefly to starvation, that is, disappearance of respirable material. In the case of the leaves used in the experiments on photosynthesis, the loss of material by respiration was more than made good by assimilation. It must, therefore, be concluded that the extent of the increase in "apparent" assimilation that could be attributed to decrease in respiration is most probably less than 0·5 units. In all cases, the increase of assimilation was of a considerably greater magnitude.

Evidence that the leaves respired normally in the photosynthetic apparatus in the light is afforded by the fact that they appeared quite normal at the end of the experiments, while some younger leaves which were used, but which failed to put out sufficient oxygen, died as a result of the anærobic conditions. These injured leaves developed the characteristic odour of the pod of the French bean, an odour absent from the normal leaf.

From the above consideration, it would appear that the inevitable conclusion to be drawn is that this increase in assimilation with progress of time cannot be due to the factors just examined, but must be attributed to increase in some "internal" factor other than chlorophyll. This aspect of the matter will be further analysed in the next section.

SECTION II.—*The Bearing of these Results on the Conception of the Photosynthetic Mechanism.*

If the only data available were those given in Table A, it might be assumed that some "protoplasmic" factor was developing from day to day and throughout acting as a limiting factor.

Before considering the question further, it will be well to be clear as to what is meant by a "limiting factor." A factor is said to be limiting a reaction when an increase in that factor results in an increase in the rate of the reaction. To take the case of chlorophyll and assimilation, chlorophyll is said to be limiting assimilation when an increase in chlorophyll leads to an increase in the rate of assimilation. It is conceivable, however, and even probable that an amount of chlorophyll-content which would be limiting under conditions of weak illumination would no longer be limiting under conditions of stronger illumination.

In considering a process such as photosynthesis, it is perhaps better to think of the reaction as consisting of stages such as diffusion, photochemical, and "dark" or chemical stages. Each of these stages conceivably depends upon two or more factors. For example, it is possible to think of the photochemical stage as dependent upon light intensity and chlorophyll-content. This stage then is limiting when an increase in either of these factors leads to an increase in the rate of assimilation. Similarly, the chemical stage may depend upon protoplasmic development and upon temperature, and consequently a degree of protoplasmic development which is limiting at a lower temperature, may cease to be limiting at a higher temperature. Further, a factor such as temperature may affect more than one stage of the process.

This analysis of the process into stages emphasises the importance of due consideration being given to such conditions as illumination and temperature before making the statement that internal factors, such as chlorophyll or those of a protoplasmic nature, are limiting. Such considerations are not always given.

On the other hand, if, under constant conditions of illumination and temperature, assimilation increases without a corresponding increase in an internal factor, such as chlorophyll, then that factor is not limiting the rate of reaction under these conditions. Such a conclusion has been arrived at with regard to chlorophyll as a result of the considerations set forth in Section I.

To elucidate further the nature of the limiting factor in the young leaves, the effect of reduced illumination on the rate of assimilation was tested. This was done on six different dates by increasing the distance of the lamp from the chamber from 6 cm. to 12 cm. The results are presented in Table B.

Table B.—Effect of Reduction of Illumination on Photosynthetic Activity of incompletely Greened Young Leaves of *Phaseolus*.

Expt. No.	Day from sowing.	Assimilation in cubic centimetres of oxygen.		Temperature.
		Lamp at 12 cm.	Lamp at 6 cm.	
III	9th	—	0	
	10th	3·3	5·0	12°–13° C.
	11th	4·3	10·4	13°–14·5° C.
IIIA	12th	3·9	9·2	14°–15° C.
IV	13th	3·3	6·6	12·5–14° C.
	14th	3·7	7·8	12° C.
VI Leaves normal green.	17th	5·0	12·0	14·5°–15·5° C.

The exact ratio of the assimilation of the two different light intensities is of no great significance for our analysis. It was proved by a number of experiments that with fully-greened leaves the temperature, when about 15° C., was limiting when the lamp was 6 cm. distant, and consequently the assimilation values given for the fully active leaves under these conditions of illumination will be dependent upon the temperature when the latter is at or below 15° C.

If the assumption that the "internal" factor which is limiting the photosynthetic process in the early stages is purely of a protoplasmic nature, that is, one involved in the chemical or dark stage of the process, be correct, then as the following consideration shows, reduction of illumination should not have the effect observed.

The maximum value of the assimilation for the fully-greened leaves, with the lamp 12 cm. distant, is 5. The assimilation is limited to this value in this case by light, that is, by the photochemical stage, since an increase of the intensity of illumination leads to an increase in assimilation. If the assimilation in the early stages of the young leaves were limited by a "protoplasmic" factor only, then whenever this factor determined the assimilation of five or more units when the lamp was 6 cm. distant, the assimilation should not be reduced below the value of five when the lamp is removed to 12 cm., since the photochemical part of the mechanism is assumed to allow of this value. As this is not the case, the internal limiting factor, which determines the assimilation in the early stages of the young leaves when the lamp is 12 cm. distant, must be concerned with the photochemical part of the mechanism.

To consider the question from another point of view, in all cases with the lamp 12 cm. distant the assimilation is limited by the photochemical stage, since an increase in the intensity of illumination results in an increase of assimilation, and consequently as the earlier stages give a smaller value than that given by the fully active leaves under the same conditions of illumination, we must conclude that this smaller value is to be attributed to an internal factor concerned with the photochemical part of the photosynthetic mechanism. This limiting factor increases its potentialities from day to day, as shown by the increasing values of the assimilation when the lamp is maintained at 12 cm.

The question as to whether the smaller values of assimilation in the earlier stages under the conditions of higher illumination were due to this same limiting factor could have been decided by testing whether these smaller values could have been increased by increasing the intensity of the illumination still further. Had the limiting factor been purely of a photochemical nature the assimilation would have increased with increasing intensity of illumination until the maximum of about 12 units, determined by the chemical stage, had been reached. The light intensity at our disposal was not sufficiently great to decide this question.

Speculation as to the nature of this factor is very attractive, but before it can be done with advantage more knowledge is required. With certainty it can be said that, although it is involved in the photochemical stage, it is not chlorophyll alone, since it increases in value without increase in chlorophyll-content.* Further, the degree of development of this factor is primarily dependent upon age and time after greening, as shown in Section I. Consequently the indications seem to be that this factor depends upon: (1) chlorophyll, since without chlorophyll the photochemical part of the photosynthetic mechanism is absent, or practically so; (2) some other factor, working in correlation with chlorophyll, which factor is dependent upon age, as very young leaves, no matter what their chlorophyll-content, cannot function as assimilating organs (see Table VII in Appendix). Further evidence as to its nature is not sufficiently definite to warrant more discussion.

SECTION III.—*A Consideration of the Results obtained by Irving and by Willstätter on the Relation of Greening to Photosynthesis, and an Explanation of the Apparent Contradiction.*

Irving, in 1910, working with F. F. Blackman, came to the conclusion that the development of photosynthetic activity in the young leaves of seedlings lagged behind greening. In other words, as the present writer's experiments show, the development of photosynthetic activity is dependent upon some factor other than chlorophyll. Irving's experiments were carried out with a small carbon dioxide supply, namely, that of respiration. She found that, under these conditions, the young leaves were unable to reduce measurably their carbon dioxide output until after they had reached an advanced stage of greening. The illumination used was greater than that required by normal leaves to utilise the whole of their respiratory carbon dioxide.

Willstätter and Stoll,† in 1915 working under what appeared to be similar conditions, found that leaves, which had developed only a very small portion of their normal chlorophyll-content, were able to utilise for assimilation the whole of their respiratory carbon dioxide. This result being in direct contradiction to that obtained by Irving, they sought, without success, for some explanation.

The only difference of procedure which they consider is, that in their experiments they took the temperature of the leaves, and thus were able to avoid an increased leaf-temperature on illumination, whilst Irving took the temperature of the bath in which the leaf-chamber was placed, and consequently the leaf-temperature was increased on illumination. Willstätter has shown in other experiments that leaves give up carbon dioxide when the temperature is raised. This, however, continues for about an hour only. A consideration of the small amount of carbon dioxide in the air current, the small change of temperature, and the duration of Irving's experiments, shows that this source of error is negligible. Willstätter also agrees that this detail in Irving's method of experimentation cannot be the cause of her results being different from his own.*

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* It may be as well to note that there is no definite evidence as to the nature of the green pigment in young leaves, as Willstätter's measurements of chlorophyll are colorimetric not chemical, whilst other writers refer to such substances as proto-chlorophyll. In this paper the term chlorophyll is used for the green pigment, whatever its nature.

† Willstätter, R., and Stoll, A., "Untersuchungen über die Assimilation der Kohlensäure," 'Ber. Deut. Chem. Ges.,' vol. 48, pp. 1540–1564 (1915).

Under conditions of more concentrated carbon dioxide supply, he found that, with increasing chlorophyll-content, the leaves showed increasing assimilation. The following Table is an extract from his results obtained with *Phaseolus*:—

Table C.—Experiments of Willstätter and Stoll on Photosynthesis with Etiolated *Phaseolus*.

Carbon dioxide 5 per cent; illumination 48,000 lux; temperature 25° C.

Exposure to light for greening.	Colour of leaves.	Chlorophyll per 10 grm. fresh weight.	Assimilation CO_2 per 10 grm. fresh weight per hour.	Assimilation number.
		mgrm.	grms.	
—	Yellow	<0·1	0·014	>70
Six hours	Green-yellow	0·6	0·080	133
Two days	Yellow-green	8·0	0·172	24
Four days	Grass green	15·6	0·204	13·3
Grown in light;	normal green	18·6	0·174	9·4

Jørgensen and Stiles,† in their review of the literature on carbon assimilation, agree with Willstätter that the latter's results show, contrary to Irving's conclusion, that, during the development of chlorophyll, it is this factor which is limiting the assimilation. They suggest that possibly Irving's results were due to light being limiting.

The only grounds they give for the conclusion that Willstätter has shown chlorophyll to be the limiting factor is the fact that the partially greened leaves have high assimilation numbers, that is, high compared with normal leaves. Consideration, however, indicates that the only conclusion to be drawn is that, with increasing chlorophyll-content, the assimilation does not increase proportionately; constancy of assimilation number, with rising chlorophyll-content, would be a nearer indication of chlorophyll being limiting. In no way can it be said that Willstätter's results prove chlorophyll to be limiting in the early stages. The increase in assimilation, with increase in chlorophyll-content observed, may be due to increase in other internal factors uncontrolled and not brought into account. This point will be taken up again later.

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* Willstätter, R., and Stoll, A., 'Untersuchungen über die Assimilation der Kohlensäure,' Berlin, 1918, p. 131.

† Jørgensen, I., and Stiles, W., "Carbon Assimilation," 'New Phytologist,' vol. 15, p. 184 (1916).

To return to Irving's experiments, and the suggestion that here light was the factor limiting the assimilation, there would appear to be no grounds for this suggestion. Jørgensen and Stiles state that Irving exposed her plants to the feeble light of a north window. Reference to her paper, however, will show that such was the case in her two preliminary experiments, but that in her critical ones she used two Keith high-pressure burners. Further, since she obtained the same results with feeble light as with strong light with leaves which had developed an almost normal amount of chlorophyll, it may be concluded that the intensity of illumination had no great effect on the results.

There still remains the task of explaining the difference between the results obtained by Irving and by Willstätter under similar conditions. At first, it seemed possible that the difference might be due to the fact that, except in her first experiment, Irving's leaves greened after being cut from the plant, and that they greened in an atmosphere where the carbon dioxide was removed as soon as formed. A comparison of leaves, which had developed their chlorophyll while attached to the plant and in ordinary air, with those which were cut and exposed to light in an atmosphere deprived of carbon dioxide, showed that there was no essential difference between their rates of assimilation.

In Willstätter's earlier account of his work he gave no record of the history of his leaves, but in his 1918 publication he states that his experiment, which was done under similar conditions to those of Irving, was performed with *Phaseolus* leaves which were exposed for six hours on the 14th day from sowing, when the shoots were about 12 inches high, and that the assimilation was measured on the following day. Irving's material, however, was taken when the shoots were only 4 to 5 inches long, that is, five or six days from sowing. Further, Irving measured the assimilation directly as the leaves greened; not 24 hours afterwards. The writer's experiments show that age and lapse of time after greening are, however, just the most important factors, and, consequently, it seems safe to conclude that the discrepant results are due simply to this, that Irving worked with very young leaves while Willstätter used distinctly older material. The experiments recorded in this paper pass from the early phase where there is distinct chlorophyll and no assimilation to the later phase where, with still little chlorophyll, the photosynthesis is quite large.

Irving's experiments also show that the assimilatory power goes on developing, though further greening is arrested by darkness, a point demonstrated by the writer's experiments. For example, in her second experiment, although, after having developed a considerable tint of green, the leaves showed no reduction of respiratory carbon dioxide, yet after the lapse of 18 hours, though further greening was inhibited by darkening overnight, they showed definite signs of photosynthesis on lighting again.

The Photosynthesis of Young Leaves Developing on Mature Plants.

A consideration of Willstätter's work on developing leaves of trees in spring and on the leaves of golden varieties very poor in chlorophyll adds further support to the conclusions drawn above.

Table D.—Relation of Photosynthesis to Amount of Chlorophyll in Different Types of Leaves of *Quercus Robur* (Willstätter and Stoll). Conditions of experimentation the same as in Table C.

Date.	Description.	Chlorophyll-content.	Assimilation.	Assimilation number.
May 2	Normal young	6·6	0·072	10·9
„ 20	„ „	8·6	0·136	15·8
June 9	„ older	21·6	0·194	9·0
July 20	„ „	25·0	0·196	7·8
„ 18	Golden variety	1·9	0·103	55·0

Note.—Chlorophyll-content and assimilation are given in same units as in Table C.

In considering these results it will be as well to bear in mind the conclusions arrived at previously as to the interconnection of external conditions and internal limiting factors.

Since the two older samples of green leaves give the same assimilation with different chlorophyll-content, it may be assumed that, under these experimental conditions, the photochemical stage is not limiting. Further evidence is supplied by the fact that other experiments of Willstätter show that normal green leaves at 25° C. reach their maximum assimilation with half the above light (about 24,000 lux). That the assimilation of the golden variety is limited by the photochemical stage is indicated by the fact that the assimilation, with the smaller chlorophyll-content, is less than that of the normal leaves, and that even at 48,000 lux the leaves show no signs of having reached their maximum of assimilation. Most probably, with still further increase of illumination and the temperature still 25° C., they would reach the same maximum as the green leaves, provided the chemical stage allowed of such activity.

It is interesting that, when through weak illumination, the photochemical stage is limiting in both the mature green and the golden leaves, the assimilation is not proportional to the chlorophyll-content, the ratio of the former to the latter being greater in the golden variety.

These facts may be expressed as follows in terms of Willstätter's assimilation number. With the temperature at 25° C. the assimilation number of both types of leaves increases with increase of illumination from zero to 24,000 lux, since the assimilation increases without change in the chlorophyll-content. Throughout this range the assimilation numbers of the golden leaves are much greater than those of the normal green leaves. On further increasing the illumination beyond 24,000 lux, the assimilation number of the green leaves remains constant, but that of the golden variety continues to increase until the maximum of assimilation is reached at a point where the chemical stage becomes limiting. Since, under all conditions of illumination, the assimilation numbers of the golden leaves are higher than those of the green, mere comparison of assimilation numbers gives no indication as to what stage or factor is limiting. Fuller consideration of illumination and chlorophyll-content such as that given above is necessary.

Willstätter comments upon the similarity of his etiolated leaves of small chlorophyll-content to the golden varieties, in that both have high assimilation numbers. This similarity is not surprising, since both types of leaves are more or less fully developed except for small chlorophyll-content, and since at 25° C. and 48,000 lux the chlorophyll in fully-greened leaves is greatly in excess of the amount required for full assimilatory activity. This similarity, however, does not warrant any conclusions such as those drawn by Willstätter as to the nature of the limiting factor or stage in his etiolated leaves.

In the case of Willstätter's etiolated leaves there are no results for material which is definitely comparable in protoplasmic development, as there are in the case of the mature green and the mature golden leaves considered above. In the earlier stages of development of leaves a difference in the age of a day may represent a considerable difference in the degree of protoplasmic development. Nor are there results for identical material under different conditions of illumination, as is the case in our experiments.

An analysis of Willstätter's results for young spring leaves gives definite evidence that chlorophyll is not here the factor limiting the assimilation. First, since in the youngest sample with a chlorophyll-content of 6·6 the assimilation is only 0·072, whereas in the golden variety the assimilation is

0·103 with a chlorophyll-content of only 1·9, the indications are against chlorophyll being limiting in the spring leaves. Secondly, these indications are borne out by the fact that young leaves 18 days older than the first sample exhibit an increase of assimilation of about 100 per cent. with an increase in chlorophyll-content of only 30 per cent. Here the assimilation number has increased with increase of chlorophyll-content; a comparison of mature golden and mature green leaves shows the opposite relation between assimilation number and chlorophyll-content. It is impossible to say in this case whether the limiting factor is involved in the photochemical or the chemical part of the mechanism. If in both cases of spring leaves the external limiting factor be temperature, as it is in the mature leaves, then the internal limiting factor would be concerned with the chemical part; but if, on the other hand, light be the external limiting factor, as it is in the leaves of the golden variety, then the internal factor would be concerned with the photochemical part.

It has thus been shown that there are three lines of evidence indicating that chlorophyll is not the limiting factor in the young stage of leaf development. In Irving's experiments the chlorophyll continued to increase, to a certain point, without any sign of assimilation; in the case of Willstätter's young leaves, as in the case of those of Irving, when they once reached the stage of beginning to assimilate, the assimilation increased out of proportion to the increase in chlorophyll; and thirdly, in the writer's experiments, the assimilation increased with no increase in chlorophyll.

Summary.

(1) The development of a leaf begins with a phase in which it has no photosynthetic power. The development of this power is shown to lag behind greening, so that a young green leaf may exhibit slight or zero photosynthesis. This means that photosynthetic activity demands the development of some internal factor other than chlorophyll. The photosynthetic potentiality of this factor rapidly increases with age, day by day, whether the leaf is in the light or in the darkness, and even though there is no concurrent increase in the amount of chlorophyll.

(2) It has been possible to demonstrate this by keeping the leaf when lighted for photosynthesis in an atmosphere of hydrogen with only a trace of oxygen, in which condition there is no increase in the initial amount of chlorophyll.

(3) The photosynthesis is measured in an atmosphere of hydrogen, to which carbon dioxide has been added. The partial pressure of the oxygen

produced is kept extremely low by being quickly removed and estimated by a new method due to Dr. F. F. Blackman.

(4) If a leaf is cut from a seedling (*Phaseolus*) in the dark at an early stage and partially greened by exposure to light in air, its photosynthetic activity will be very small or nothing, while if exactly the same procedure is repeated with a similar leaf from the same plant a few days later, quite strong photosynthetic activity will be found.

(5) This alteration of the internal factor with age explains why Willstätter has failed to confirm the observations published by Miss Irving in 1910, proving that young leaves of a full green colour might yet possess no power of photosynthesis. Miss Irving used very young leaves, while Willstätter happened to use leaves cut from plants some nine days older. In the present research the results of both observers have been obtained in succession on the same plant.

(6) The conception of the photosynthetic process as consisting of stages such as diffusion, photochemical, and chemical stages necessitates the consideration of external factors when stating that an internal factor is limiting.

(7) Consideration of the results here presented shows that the activity of the photochemical part of the photosynthetic mechanism in young seedling leaves as compared with that of more mature leaves is in some way limited, but apparently the photochemical part depends for its intensity not only upon chlorophyll but also upon some other factor. This factor increases with age during the early stages of leaf development.

(8) Willstätter's conclusion that chlorophyll was limiting in his results with etiolated leaves is based upon insufficient evidence. Full analysis of his results with young spring leaves shows that chlorophyll is not limiting, just as it is not limiting in Miss Irving's experiments, and in the experiments described in the present paper.

APPENDIX.—*Assimilation Experiments.*

Each Table refers to the experiment of that number in the text. The first measurement of assimilation was made immediately after greening, except in the "A" experiments, when the leaves were kept in the dark during the period between the time of greening and measurement of assimilation. During the periods between the estimations of assimilation the leaves were kept in the dark in air at the temperature of the laboratory (14°–15° C. in Experiments I–VI) except in Experiment IA when the temperature was 24°—25° C. A preliminary of about one hour in the apparatus, prior to estimation of assimilation, was allowed.

Tables I–VII.—*Phaseolus vulgaris.*

Date.	Temperature, C.	Distance of lamp from chamber in cm.	Oxygen per grm., dry-weight per hour, c.c.	Duration of estimations in hours.	History of material.
Table I.					
17.x.19	14°–15°	6	1·2 1·3	5½	Sown 8.x.19, at 25° C. Cut at 9 P.M., 16.x.19, and exposed to lamp at 14°–15° C. for 13 h. Leaf colour, green-yellow.
18.x.19	14°–15·5°	6	2·4 2·7	3½	
20.x.19	15°	6	7·2 6·8	2¼	
21.x.19	15°	6	7·5 6·7	4	
Table IA.					
18.x.19	15°	6	4·1	2¼	As in Table I.
19.x.19	13°	6	5·2 5·7 5·7	4¼	
20.x.19	15°	6	6·5 6·8	3	
Table II.					
22.x.19	15°–16°	6	7·7 7·8	6	Sown 8.x.19, at 25° C. Cut at 7 P.M., 21.x.19, and exposed to lamp at 14°–15° C. for 15 h. Leaf colour, green-yellow.
23.x.19	15°–16°	6	8·5 10·0	5	
24.x.19	15°–16°	6	10·2 9·8	3	
Table IIA.					
24.x.19	15°–16°	6	11·0 10·4	2½	As in Table II.
Table III.					
4.x.19	12°–13°	6	0	7	Sown 26.ix.19, at 25° C. First six days from sowing at 25° C., remaining day at 14°–15° C. Cut at 10.45 A.M., 3.x.19, and exposed diffuse daylight for 8 h. and 17 h. lamp. Leaf-colour, yellow-green.
5.x.19	12°–13°	6	4·8 5·1	3	
5.x.19	12°–13°	12	3·3 8·6	3	
6.x.19	13°–14·5°	6	10·3 11·0	4	
6.x.19	13°–14·5°	12	4·3	2	

Tables I–VII—*continued.*

Date.	Temperature, C.	Distance of lamp from chamber in cm.	Oxygen per grm., dry-weight per hour, c.c.	Duration of estimations in hours.	History of material.
Table IIIA.					
7.x.19	14°–15°	6	9·1 9·2	3	As in Table III.
7.x.19	14°–15°	12	3·9	1¾	
Table IV.					
8.x.19	12·5°–14°	6	4·4 6·7 7·0	4½	Sown 26.ix.19, at 25° C. First six days from sowing at 25° C., remainder at 14°–15° C. Cut at 9.30 P.M., 7.x.19, and exposed to lamp for 13 h. Leaf colour, green-yellow.
8.x.19	12·5°–14°	12	3·3	3	
9.x.19	12°	6	7·6 7·9	3	
9.x.19	12°	12	3·7 3·8	2¼	
10.x.19	11°	6	8·7 9·6	3	
Table IVA.					
10.x.19	11°	6	7·3	2	As in Table IV.
11.x.19	10·5°	6	7·0 7·0	4	
Table V.					
15.x.19	14°	6	11·9 12·1	2	Sown 26.ix.19. Plant exposed to diffuse light on morning of 11.x.19, until 15.x.19. Leaves, normal green.
Table VI.					
25.x.19	14·5°–15·5°	6	11·5 12·3	1½	Sown 8.x.19. Plant exposed to diffuse light, 19.x.19, until 25.x.19. Leaves, normal green.
25.x.19	14·5°–15·5°	12	5·0 5·0	3½	
Table VII.					
21.vi.19	17·5°–18·5°	6	0	10½	Sown 14.vi.19. Exposed 19.vi.19, until 21.vi.19; diffuse light. Colour, grass green. Leaves dead at end of experiment.
22.vi.19	17°	6	0	1	

Tables VIII–XA.—*Avena Sativa.*

Date.	Temperature, C.	Distance of lamp from chamber in cm.	Oxygen per grm., dry-weight per hour, c.c.	Duration of estimations in hours.	History of material.
			Table VIII.		
19.vi.19	21°–23°	6	4·4 6·6	3½	Sown 12.vi.19, at 25° C. Exposed 18.vi.19, to diffuse daylight until grass green.
19.vi.19	21°–23°	12·5	2·8	3	
19.vi.19	21°–23°	6	14	2	
20.vi.19	20°	6	23	1	
			Table IX.		
23.vi.19	18°	12·5	1·4 1·7 2·2	2	Sown 18.vi.19, at 25° C. Grown in diffuse daylight. Colour, grass green.
23.vi.19	18°	6	4·9 7·1 10·0	6	
			Table X.		
30.vi.19	15°–17·5°	6	2·4 3·8	8½	Sown 23.vi.19, at 25° C. Exposed 29.vi.19, for 7 h. Colour, green-yellow.
1.vii.19	17·5°–19·5°	6	6·1 7·0	2½	
			Table XA.		
1.vii.19	20°–25°	6	5·2	1½	As in Table X.
2.vii.19	20°–25°	6	6·1 5·8	2¾	

Table XI.—*Vicia Faba.*

Date.	Temperature, C.	Distance of lamp from chamber in cm.	Oxygen per grm., dry-weight per hour, c.c.	Duration of estimations in hours.	History of material.
8.viii.19	19°–22·5°	6	0 0·9	7½	Sown 31.vii.19. Exposed to diffuse daylight, 6.15 P.M., 7.viii.19, to 10.15 A.M., 8.viii.19. Colour, yellow-green.
9.viii.19	22°–25°	6	1·1 1·6	9	

Respiration Experiment.

Table XII.—*Phaseolus.*

Date.	Temperature, C.	CO per grm., dry weight per hour, c.c.	Remarks.
4.xi.19	11°–12°	1·40	Sown 29.x.19. Dry weight, 0·14 grm. Fresh weight, 0·74 grm.
5.xi.19	11°–12°	0·85	
6.xi.19	10°–11°	0·77	

Studies on Synapsis. I.—Oogenesis in the Hymenoptera.

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[Plates 4–9.]

For establishing a correlation between genetic and cytological phenomena, the Hymenoptera furnish a series of forms which offer fertile possibilities; the production of males from eggs which segment without fertilisation makes it possible to state definitely the point in the germ cycle at which the determination of sex is effected. While, however, much work has been done from the cytological standpoint in connection with the group, our knowledge is still inadequate with respect to those species which produce females as well as males by parthenogenesis. What little is known of this subject has been derived exclusively from a study of the polar and cleavage mitoses; and in view of the importance of treating the germ cell cycle as a whole, and also of the unfavourable material which heavily-yolked ova necessarily afford, it seemed desirable to investigate oogenesis in typical and completely agamic Hymenoptera, more especially because data relating to the maturation prophases of the female germ cells in insects are much less numerous than in the case of spermatogenesis. The original purpose of the author in pursuing the present investigation arose out of previous work on nuclear phenomena in the late ovarian oocytes of *Neuroterus,* which yielded indications of post synaptic syndesis. Agar has described a second conjugation of chromosomes after a temporary dissociation of univalents in *Lepidosiren,* unconfirmed hitherto in any other animals; and since the demonstration of such an occurrence in the oogenesis of insects might well provide a basis of reconciliation for the rival hypotheses of parasyndesis and metasyndesis (telosynapsis), the synaptic phase in the oocytes of the Hymenoptera was selected for the first of the present series of studies. In addition to the two-fold problem already stated, there have arisen several subsidiary questions in the course of the investigation, notably the peculiar character of the mitotic figure during ookinesis in certain families, and the significance of certain bodies characteristic of the Hymenopteran egg, namely, the so-called germ cell determinants and the remarkable secondary nuclei.

Wherever possible I have avoided the use of sublimate fixatives employed by previous workers. Bouin gave excellent results; but the best preparations were obtained by the use of Flemming modified to increase its penetrating power by means of a trace of urea.

1. *Cynips Kollari.*

Cynips kollari is the gall wasp which causes the exceedingly common marble gall to develop from leaf buds of the vegetative shoot on the oak. Its special interest for present purposes consists in the fact that unlike the other gall-makers which infest the oak, *Cynips* displays no alternation of sexual and parthenogenetic generations. A sexual brood is lacking in the life cycle; not only is the species exclusively agamic, but it is composed entirely of females. It has been chosen for investigation not with a view to elucidating directly the chromosome cycle of a form which produces females from unfertilised eggs, since the difficulty of inducing it to breed in captivity is too great; but it was hoped that it would assist in interpreting the phenomena which have already been investigated in another agamic Cynipid, *Rhodites.*

The somatic divisions were studied in the young pupæ in epithelia, nerve cells, developing wings and other centres of cell multiplication. The chromosomes in *Cynips* are unbent and filamentous; their number is 20, as in *Neuroterus.* The same number is found in dividing follicle cells, an anaphase of which is shown in Plate 4, fig. 2. I have not observed either asters or centrosomes in connection with the spindles of somatic figures, as illustrated in fig. 1 from the larval gut.

Early History of the Oocyte.

In the full grown larva the ovaries are small spherical bodies surrounded by a capsule of connective tissue and consisting of undifferentiated cells with granular nuclei (fig. 3). Mitoses are extremely rare; it seems that the ovaries reach this stage of development at an early date in the life of the larva, remaining in this condition until pupation. All three types of cells in the ovariole of the mature ovary of *Cynips*—follicles, nurse cells, and oocytes—originate from germ cells. Certain of the primary oogonia by a series of divisions (probably synchronous) give rise to groups of cells, which correspond to the "rosettes" described in other insects. The rosettes consisting of 16–32 cells, when division has ceased, give rise both to the oocytes and nurse cells which are not distinguishable until the nuclei have been through the initial stages of synapsis.

Differentiation of oocytes and nurse cells takes place in the young pupa before the ovaries are clearly separated into strands; and no synapsis nuclei are found in the terminal portions of the egg tubes except in very young pupæ. The cells of a fully formed rosette at first possess nuclei the chromatin of which is present in masses of probably inconstant numbers (fig. 4).

The changes which ensue preparatory to differentiation are: (1) Leptonema, (2) Synizesis, (3) Break up of the post synaptic spireme.

(1) *The Leptotene stage* is characterised by the appearance of a dense tangle of pale and very much convoluted threads (fig. 5).

(2) *Synizesis* follows immediately upon the Leptotene stage (fig. 6 *a*, *b*, *c*, *d*).

(3) *Post Synaptic spireme.*—The chromatin emerges from the synaptic knot at first as an apparently continuous spireme. This, however, is soon seen to segment into separate filaments (fig. 8). From a careful examination of a large number of nuclei seen immediately after the break up of synizesis, it is almost certain that the haploid number, *i.e.*, about ten threads, make their appearance. Immediately after this, two sorts of nuclei can be distinguished: (*a*) those in which the chromatic elements loose their visible individuality (fig. 9*a*); (*b*) others in which the chromatin threads undergo fragmentation into innumerable minute granules (fig. 7*c*). The former develop into functional oocytes, the latter become nurse cells. A large number of cells were observed which indicate (fig. 7 *a*, *b*) the possible intercalation of a diplotene stage in the differentiation of the nurse cells; at the time when the latter can be first distinguished from oocytes by their nuclear behaviour, there are no recognisable differences in the size of the cell itself or the appearance of the cytoplasm.

The nucleus of the oocyte remains in the "diffuse" stage during the entire growth period, until the end of pupal life.

The Growth Period. General Characteristics of the Egg.

The young egg during the period of growth is oval; it is not completely surrounded by follicular cells at the extremities where the cytoplasm projects into the adjacent nurse chamber entering into relation with the nurse cells. In the early growth period, before the deposition of yolk has begun, several spherical bodies of varying sizes staining with chromatin dyes are found lying in the cytoplasm; in one case two such bodies, one at the anterior end and one at the opposite pole were observed (fig. 9*b*). In other cases their number was more numerous; they appeared to increase in size in proportion as they were remotely situated from the nuclear membrane. Some sections showed minute granules lying within, upon and just outside the nuclear membrane; and the nucleus in such may show several karyosomes, so that possibly these bodies are the products of the oocyte nucleus. As in *Neuroterus* and *Andricus*, it is not possible to detect in the eggs of *Cynips* any region which can be said to correspond with the "germ-cell determinants" of *Copidosoma*. The full-grown egg, unlike the elongated type which is characteristic of the Hymenoptera, is fusiform, tapering to a fine stalk at one extremity (fig. 10).

Nuclear Phenomena in the late Ovarian Oocyte.

About the time when the egg reaches its maximum dimensions, *i.e.*, just before the imago bores its way out of the gall, the chromatin of the nucleus resolves itself into long filaments of which about 20 can be counted (fig. 11). These then become shorter and thicker, preparatory to conjugation in pairs, so that each member of a pair is in contact with its fellow by one extremity only. Since the number of chromosomes is reduced in the early oocytes of the young pupa, *it would appear that the chromosomes pair twice in the forms which are now under consideration.* Agar has described a reunion of univalents after a period of temporary separation preparatory to the formation of the maturation spindle in the spermatogenesis of *Lepidosiren*. Wilson has also stated that in the spermatogenesis of certain Hemiptera the X and Y chromosomes regularly conjugate at the poles of the spindle before the first maturation division is completed. Hegner believes that a pairing of chromosomes end to end immediately before the appearance of the maturation spindle occurs in *Copidosoma*. These bivalent elements, which are distributed at first without any definite arrangement (fig. 12), betray their double nature by a deep constriction in the middle. They soon begin to arrange themselves about a definite axis, and spindle fibres make their appearance, though no asters or centrosomes are visible. The number of doubles present at this stage is very easy to count, 10 being invariably present (fig. 13). They are now seen to array themselves in a parallel series upon the spindle with the point of contact of their univalent constituents in the equatorial plane. What follows corresponds exactly with what has been described in *Copidosoma* and *Neuroterus*. The chromosomes of the spindle come more closely into contact till they form a compact complex; the spindle disappears, the chromosome complex becomes more or less spherical, and the clear area occupied before by the nuclear sap is gradually invaded by yolk-laden cytoplasm, so that it is rendered inconspicuous (figs. 14–17). The condensation of spindles in the late ovarian oocyte has already been described in three Chalcid genera, *Ageneaspis* (Martin), *Copidosoma* (Hegner and Silvestri), and *Trichogramma* (Gatenby), in the Cynipids *Andricus* (Hegner) and *Neuroterus* (Hogben), as also in the Braconid *Apanteles* (Hegner).

The precise stage at which the nuclear membrane dissolves in the process here described is difficult to determine, owing to the sharply defined meniscus separating the clear space occupied by the nuclear sap and the surrounding heavily yolked cytoplasm. For this very reason it can be said, however, with some assurance that the spindle fibres originate in the area originally occupied by the resting nucleus. The existence of a maturation spindle in the ovarian oocyte of *Cynips* does not imply a reductive division in the formation of polar bodies. Reduction of the chromosomes in the oocytes of the agamic Spring females of *Neuroterus* occurs, but, while an abortive maturation spindle is found, no polar bodies are extruded in the female-producing ova. Possibly the same holds good for *Cynips*. Or alternatively, if polar bodies are produced, as is more usual in parthenogenetic ova, both divisions may be equational, and a subsequent disjunction of univalents may ensue. Whatever be the behaviour of the nucleus in *Cynips* after the egg is laid, it may be confidently asserted that the early history of the oocyte resembles that of other Hymenoptera parasitica, and the chromosomes, which are seen as the maturation spindle of the ovarian oocyte, are bivalent in character.

Rhodites rosæ.

Rhodites rosæ, which is responsible for the Bedeguar Rose gall on the wild rose, is too familiar to merit introduction. It has already been made the subject of cytological researches by two workers, but, since their conclusions are at variance, and do not seem to show an appreciation of the peculiar history of the nucleus in the later maturation prophases of the Cynipid egg, there is good reason for completing and criticising the accounts hitherto given.

Henking (1892) found that, in the full-grown oocytes, shortly before laying, nine chromosomes (which he concluded to be bivalent), are present. Two polar bodies are formed, and show 9–10 chromosomes, as does also the anaphase of the segmentation nucleus. According to this author, the segmentation nucleus, after a period of rest, during which its staining capacity greatly decreases, shows 18–20 chromosomes on the equatorial plate of the first segmentation division. Segmentation mitoses showed the same number. Schleip reinvestigated the cytology of the egg in *Rhodites* in 1909. He gives the number of chromosomes in the young oocyte as 10–12, and denies that those of the female pronucleus are doubled before the first cleavage, finding that the segmentation mitoses show 12 chromosomes. He concludes that there is no reduction in *Rhodites*, though polar bodies are formed, *i.e.*, the maturation mitoses are equational. In heavily yolked eggs where the number of chromosomes is small, the segmentation divisions do not afford the most profitable basis for a study of the chromosome cycle, and, unhappily, neither of these investigators has added to his work data respecting the one point which would give most weight to the results obtained, namely, the number of chromosomes in the oogonial mitoses. In the present research, the somatic and oogonial mitoses of the larval and pupal stages have been thoroughly investigated, and the history of the oocyte, from its origin to the time of laying, has been worked out for the first time.

The Somatic Mitoses.

It has already been mentioned that neither Henking nor Schleip commenced their investigations on the chromosome cycle of *Rhodites* by a study of the mitoses in the larval or pupal stages, in which the oogonia are to be found dividing. As pupation is rapidly completed in *Rhodites*, the task of finding nuclei in division during the early stages is not a difficult one. In sections cut from material fixed in the inception of pupal life, mitoses are abundant in the developing wing epithelia, nervous system, and follicle cells. In all cases the number of chromosomes is at least 18; Henking gave 18–20 for the segmentation mitoses, and an element of uncertainty must be conceded in virtue of the extremely minute dimensions of the chromosomes in *Rhodites* as compared, *e.g.*, with *Neuroterus*, or, more particularly, with *Cynips*. The existence of 18 chromosomes in the dividing nuclei of the somatic tissues is not conclusive evidence that the same number occurs in the germ cells; in *Apis*, the somatic mitoses of the male show four times the number of chromosomes displayed by the segmenting spermatogonia, and in *Neuroterus* they display the number 20 (characteristic of the female) in both sexes, while spermatogonial counts give half this number. Nevertheless, the follicle cells in the ovaries present another case. Follicular cells have frequently been described as dividing amitotically in insects; in some cases, as in the pædogenetic Cecidomyid *Miastor*, oocytes alone are truly germinal in origin. In the Hymenoptera the follicle cells divide normally, and it has been well established by the work of Korschelt (1886), Paulcke (1907), and

Marshall (1907), that all three cell elements in the ovarioles of this group—nurse cells, oocytes, and epithelia—arise equally from the differentiation of germ cells. For two reasons, then, the nitotic figures in the follicular cells of *Rhodites* demand special consideration, as indicating the true diploid number of chromosomes: (i) in the allied Cynipid genus *Neuroterus*, according to Doncaster, dividing follicle cells give the same count as dividing oogonia, and the same is seen in my own preparations of *Cynips*, set forth above; (ii) in origin they are germinal. I have figured a particularly clear anaphase of a dividing follicle cell showing 18 chromosomes at one pole, and this avoids any suspicion that both sets of chromosomes have been counted in an equatorial plate; some stress is laid upon this, since Henking's count of the segmentation mitoses is almost twice that of Schleip (Plate 5, fig. 18).

The Synaptic Phase.

In the full-grown larvæ at the end of the first season the ovaries are inconspicuous ovoid masses of cells with granular nuclei. The ovary is surrounded by a capsule of connective tissue, and its component cells are undifferentiated. As in the case of *Cynips*, the fact that mitoses do not normally occur at this stage suggests that the ovaries are formed early and pass temporarily through a period of rest, during which there is no cell division. In the following spring (and sometimes in the autumn of the first season) changes begin to occur. Oogonia begin to divide into rosettes (fig. 20). The cells of a rosette divide synchronously, as in *Apis*.

Some 25 clear anaphase plates at this stage were observed, in which it was possible to count the chromosomes without difficulty, the number being the same—18—as that in the dividing follicle cells (fig. 19). This conclusively vindicates Henking's assertion regarding the somatic number in *Rhodites*.* In early stages of ovaries at the inception of differentiation it is possible to distinguish three regions: (*a*) zone of rosettes; (*b*) zone of undifferentiated oogonia; (*c*) zone of flattened apparently vacuolated cells, which correspond to those which are found in the terminal filaments of the ovarioles of young pupæ. With the exception of the last, all cells rapidly become divided to form rosettes, and it is in the fully formed rosettes of the larva just before pupation that the synaptic stages are to be traced.

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* I find that several writers who have accepted Schleip's observations, including Miss Harvey and Cutler, states that he gives 10–12 as the number of chromosomes in the dividing oogonia. It is true that he says, in describing the segmentation divisions, "der normale Chromosomenzahl bei *Rhodites rosæ* ist also etwas 12; die gleiche Zahl müssen wir auch in den Oogonien annehmen"; but nowhere does he refer to preparations or give illustrations of oogonial mitoses—on the contrary he expressly asserts (erroneously), "die Zahl der Chromosomen in den Ureizellen ist nicht zu bestimmen."

The stages of synapsis in *Rhodites* are here described for the first time, and I am able to supply fuller details than in the case of *Cynips.* As in the latter, the preleptotene nuclei display irregular masses of chromatin. Nurse cells cannot be distinguished from functional oocytes until after synizesis, and the nuclear history of the former may be dealt with first:—

1. *Leptonema.*—The chromatin of the nucleus forms a tangle of pale convoluted threads, which are too intricately crossed to permit the possibility of counting them (figs. 21, 22).

2. *Synizesis.*—The leptotene threads increase in staining capacity and contract away from the nuclear membrane in a compact knot (figs. 23–25).

3. *Post Synaptic spireme.*—As synizesis dissolves, a spireme emerges, which breaks up into pachytene filaments (figs. 26, 27), of which in over 20 counted about 9 are clearly present.

4. *Diplotene.*—Immediately after the foregoing, the pachytene threads are seen to be split lengthwise; these double threads shorten up into rectangular masses of ragged outline, which are still evidently double. These masses break up into minute granules which are dispersed throughout the nucleoplasm of the fully-formed nucleus of the nurse cell (figs. 28–30).

The foregoing account shows striking similarity to Dederer's recent work on the moth *Philosamia,* in which the history of the nurse cells is almost identical.

The young oocyte nucleus, after passing through the stages of leptonema, synizesis, and pachynema, begins to show a greatly diminished staining capacity; it becomes difficult to trace separate threads, though it seems likely from some preparations that the diploid number is present. A striking fact is that the diminution of the staining capacity of the nucleus coincides with the appearance of nuclear granules on the nuclear membrane within the nucleus and in the surrounding cytoplasm, thus confirming the opinion previously expressed that the chromatin-staining granules in the young egg of *Cynips* are of nuclear origin (fig. 30). During the greater part of the growth period the nucleus remains in the "diffuse" stage, *i.e.*, the visible identity of the chromatin elements can no longer be traced with certainty; but in the very late pupal ovary nuclei can be found in which long thin filamentous chromosomes are present, staining deeply; their number is about 18.

The oocyte is oval during the growth period. The cytoplasm at first projects into the adjacent nurse chamber; but by the time the imagines emerge the egg has reached the full size, the follicle is complete and reduced to a thin lamella, while the nurse cells are no longer seen. The egg then is elongated, slightly pointed at each end, and tapering off into a stalk at the anterior extremity (fig. 31). At the posterior pole, in preparations that have been decolorised so as to remove the stain from the yolk, there is seen to be a region of deeply staining granules. The presence of this body was originally noticed by Weismann (1882), who applied to it the term "Furchungskern"; needless to say, it is not the cleavage nucleus, nor is there any reason to suppose that it is of nuclear origin. Tanquary, curiously, applied the same term to a similar collection of granules in the egg of the ant *Camponotus*. Hegner, who has not examined the egg of *Rhodites* himself, has suggested that these granules are of the nature of what he calls "germ-cell determinants." Their later history has not yet been followed, nor has that of the more definitive body described in the Cynipid *Diastrophus;* and in view of the conflicting accounts that have been given by Martin, Silvestri, Hegner, and others as regards both origin and fate of the "germ-cell determinants" in Hymenoptera, it might be advisable for the present to refrain from using this term until a more precise acquaintance with their genetic and developmental significance is forthcoming.

Nuclear Phenomena in the Late Ovarian Oocyte.

About the time that the gallfly emerges, the oocyte nucleus is large and conspicuous, occupying a position about midway from either pole. It has been said that, towards the end of the growth period, the chromosomes regain their visible identities, and appear in the diploid number (fig. 32). What follows has been described by Schleip, thus: "Zuerst sind sie Fädchen mit den bekannten Knotchen-förmigen Verdickungen und einer allerding nicht sehr deutlichen Längspalte. Der relativ kleine Nucleolus bleibt erhalten bis etwas zu der Zeit wo die Kernmembran sich auflöst. Dann werden die Chromosomen dicker und erscheinen im Präparat schliesslich als unregelmässig geformte Klümpchen. Einige von ihnen sind deutlich ringförmig."

There are two points in this short account which compel comment. First, the chromosomes are not at this stage irregular. Originally in the form of filaments which are clearly double, like those of *Cynips* or *Copidosoma*, they shorten up into dumb-bell shaped bodies, that still preserve their bivalent character (figs. 33, 34). The only conclusion that can be drawn from Schleip's figures is either that they are very diagrammatic, or that they illustrate preparations imperfectly fixed. In the second place, none of the chromosomes are ring-shaped; at this stage *ring-shaped* plasmosomes are frequently seen as in *Synergus*;* and, as earlier described, the plasmosome of the preceding stage is seen to be vacuolated in preparation for its dissolution into ring-like bodies. This probably accounts for the fact that Schleip is able to count from 10 to 12 chromosomes (doubles), whereas I have never counted more than nine, thus agreeing with Henking, who also regards these chromosomes as bivalents. Those who have studied the late maturation prophases of the Cynipidæ would anticipate from these phenomena the subsequent formation of an abortive spindle such as has been demonstrated in *Andricus*, *Cynips*, and *Neuroterus*. Neither Henking nor Schleip have followed up the succeeding stages in detail. According to the former, who gives a much more thorough survey of the data, "lange bleiben die Chromosomen jedoch nicht von einander getrennt. In den etwas älteren Eiern bemerkt man nämlich dass deren mehrere zusammen zu treten beginnen, entweder zu Klumpen, oder auch Ketten. Je mehr das Ei reift, um so mehr schreitet dieser Process fort, so das schliesslich alle Chromosomen zu einem gemeinsamen Ballen vereinigt sind." Later he observes "der farblose Kernraum an Volumen eingebüsst hat." Henking does not mention the presence of spindle fibres, but, in reality, a very clear spindle is (figs. 35–37) formed, without either aster or centriole, and upon this spindle the bivalent chromosomes arrange themselves in a parallel series in the equatorial plane.

The mitotic figure now passes through the stages of condensation; the chromosome complex (figs. 37, 38), at first elongated with respect to the axis of the spindle, becomes spherical; the spindle itself disappears, and the so-called nucleus becomes lost in the surrounding yolk. One further point that may be mentioned is that the karyosome disintegrates into a number of granules that surround it.

The discovery of a precociously formed and interrupted maturation spindle in late ovarian oocytes of *Rhodites* is of some cytological interest, as throwing light on the character of the peculiar mitotic phenomena of ookinesis in certain Hymenoptera, and confirming the conclusions I have elsewhere expressed in regard to the figures originally described by Hegner, based on previous work on *Neuroterus*; for, according to Henking's account the preparation of the polar nuclei is very much like the corresponding process described by Doncaster in the eggs of *Neuroterus* and of a type differing widely from the ordinary form of mitosis. An atypical formation of polar bodies in *Trichogramma* and *Ageniaspis*, which also show abortive spindles in the ovarian egg, has been referred to earlier. Gatenby describes the process loosely as "amitotic." Martin says that the mass formed by the condensation of the maturation spindle "divides in polar body formation without the presence of spindle fibres or asters." It may be confidently predicted that the formation of polar bodies by a similarly atypical procedure will be demonstrated by subsequent research in other forms, where the history of the egg nucleus shortly before deposition is comparable.

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* *Vide infra.*

I have not investigated the maturation of the egg in *Rhodites*. The crucial difference between the rival conclusions of the two authors referred to lies in their statements regarding the segmentation mitoses, which, according to Henking display 18–20, according to Schleip 10–12 chromosomes. Concerning the latter view, I would here submit (1) that Schleip failed to appreciate the double nature of the chromosomes which he counted (together with karyosomes) in the oocyte nucleus; (2) that a study of the nuclear divisions in the segmenting egg does not provide such a satisfactory basis for counting the chromosomes, when small and numerous, as do the oogonia and somatic tissues of the pupa; (3) it is easier to underestimate the number of chromosomes than to count more than are actually present; (4) were the chromosome cycle in *Rhodites* such as set forth by Schleip, it would be remarkably unlike that of other Cynipids, notably, *Cynips kollari*. Furthermore, the last-named investigator seems to me to have approached the subject in an attitude suggestive rather of propaganda than of scientific impartiality, and appears over-anxious to show that Henking's conclusions are mistaken, because of their similarity to those of Petrunkewitsch with respect to the drone bee. He criticises the alleged doubling (*i.e.*, disjunction) of chromosomes during interkinesis, on the ground that it does not fall in with accepted views regarding the persistent individuality of the chromosomes; but the facts of synapsis alone vitiate such an objection; and the fact that he finds only six chromosomes in the blastoderm stage confirms my belief that his preparations were not satisfactory. The events of the chromosome cycle in *Rhodites*, then, may be summarised as follows: (1) the somatic and oogonial mitoses show 18 chromosomes; (2) parasynapsis probably occurs in the young oocyte;* (3) at the conclusion of the growth period the diploid number reappears; (4) these filaments then conjugate, so that the full-grown oocytes show nine chromosomes, the origin of which is betrayed by their double character; (5) the haploid number of bivalents is arranged on an asterless spindle, which passes through the stages of condensation; (6) the segmentation nucleus, according to Henking, contains

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* It is difficult to interpret the restoration of the diploid number by the longitudina splitting of the pachytene threads without assuming that the latter arise by the side-by-side union of leptotene threads.

nine chromosomes, which number is doubled prior to the first segmentation division.

Significance of the Polar Divisions.

The character of the chromosome cycle in the production of drones in the Hymenoptera may now be regarded as settled. In those completely parthogenetic species which produce thelytokous ova without impregnation our knowledge is scanty, but it seems clear that the same uniformity does not prevail; the only really satisfactory account is that of Doncaster who investigated the agamic generation of *Neuroterus*, in which form there are no polar bodies extruded by those eggs which develop into females. This is not, however, typical of the Hymenoptera where in several cases agamic female-producing eggs are known to form two polar bodies, as in the case of *Rhodites, Pœcilosoma* and *Crœsus.* These forms also contrast with the cytological behaviour of female-producing eggs among parthenogenetic organisms belonging to other groups, where as a rule females are produced from eggs which extrude only one polar body by an equational division.

The question now arises; what relation have the chromosomes that separate during the interkinesis preceding segmentation to one another? There seem to be two possible ways of interpreting Henking's "Verdoppelung": (1) the polar mitoses are both equational, and the doubling of the chromosomes in the female pronucleus is a disjunction of bivalents; or (2) the chromosomes which pair in synapsis are equivalent daughter halves of a single chromosome which has divided before segmentation begins.* Apart from the question of sex determination, either hypothesis is equally acceptable, an objection, however, to the first being the extraordinary similarity of the separation of polar nuclei in female-producing eggs of *Rhodites* and male-producing eggs of *Neuroterus,* which are probably reduced qualitatively and quantitatively. The second hypothesis in the case of *Rhodites* offers no explanation of the occasional appearance of males, and does not fall into line with conclusions regarding sex-determination based upon the study of other Hymenoptera. Nachtsheim, to account for the difference of sex in fertilised and unfertilised eggs of the bee, has put forward the theory that, of the 32 chromosomes in the oogonial mitoses of *Apis,* 30 are somatic and two are X-chromosomes; at maturation reduction occurs, so that the female pronucleus should have the constitution 15-X, thus a virgin egg would have a single sex chromosome, while a fertilised egg will have two. The sexual differentiation in the chromosome cycle of *Apis* is by this means made to agree with the case of those insects in which maleness is associated with a single X-chromosome and femaleness with a pair; as, however, there are no visible cytological peculiarities in these hypothetical X-chromosomes, and since moreover the question of sex-limited inheritance in the Hymenoptera is a subject which has not hitherto been investigated to my knowledge, it is only possible to accept Nachtsheim's suggestion with some reservation.

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* Nachtsheim's work on the segmentation of the drone eggs of the bee renders such a hypothesis not improbable *a priori.*

3. *Synergus Rheinhardii.*

In rearing *Cynips* a large number of individuals of *Synergus rheinhardii* and the smaller allied species *S. melanopus* emerged from the galls in which they pass their larval and pupal phases as inquilines. A study of the eggs of the inquiline *Synergus rheinhardii* revealed certain points and led to conclusions which seem to merit record.

The Oosoma.

In several respects the egg of *Synergus* resembles closely that of *Trichogramma* and *Copidosoma.* Like these it possesses when it has attained its full size a well defined body at the posterior pole staining deeply with chromatin dyes. This corresponds to what Silvestri originally called the "nucleolo," and Hegner the "Keimbahn" or "germ-cell determinants." Both of these authors originally believed that it arose from nuclear material. In view of its subsequent distribution in certain forms among the germ cells this view is a tempting one to those who advocate the doctrines of Weismann in their extreme form; but it has been shown by Gatenby in *Trichogramma,* Martin in *Ageniaspis,* and since reported by Hegner and Silvestri in *Copidosoma* that it is derived from the cytoplasm. In *Synergus* as in *Trichogramma,* the "germ-cell determinant" arises "as a cloud of granules which make their appearance spontaneously in the cytoplasm . . . become more and more heavily staining and denser until the determinant resembles a spherical ball at the end of the egg" (figs. 39–41). Further, in *Synergus* as in *Trichogramma,* the reaction of this body with respect to Gilson, Petrunkewitsch, Flemming, and other acetic fixatives employed shows that it is neither wholly nor partially of mitochondrial matter as Martin believed. The fact that the "germ-cell determinant" is a cytoplasmic inclusion provides confirmation of the view of organ-forming areas in the cytoplasm of the egg. Gatenby has suggested that the function of the determinant is nutritive, preventing the germ-cell nuclei from being exposed to the uncertain conditions existing elsewhere in the segmenting egg and embryo. This might be correlated with its more frequent occurrence in parasitic forms. Such bodies do not occur in many gallmaking Cynipids, egg *Neuroterus, Cynips,* and *Andricus:* there is, however, a well defined structure at the posterior pole of the egg in the blackberry knot gall-fly, *Diastrophus.* Since the fate of the "germ-cell determinant" has only been worked out in a few cases, it would appear advisable to adopt for the present Silvestri's newly proposed term "oosoma," or if the rule as to precedence is rigidly enforced, alternatively, let it be still called the "polar disc." This would obviate the drawback of being committed to any particular theory as to its physiological or genetic significance in the developmental process.

The Secondary Nuclei.

Gatenby (1918) has noticed in the egg of *Trichogramma* the presence of "large spherical granules" staining deeply with iron hæmatoxylin. They were also found in *Cynips* and smaller and more numerous bodies of similar origin in *Rhodites*, where they have been shown to be of nuclear origin, their appearance being correlated with the diminution of staining capacity exhibited by the nucleus during the growth period. Such granules are found at all stages after synapsis in *Synergus.* In the full grown egg little groups of granules are sometimes recognised in the peripheral region; these appear to be the result of fragmentation of the larger nucleoli. During the growth period the nucleus is seen to contain numerous granules of various sizes.

In addition, true secondary nuclei make their appearance in the peripheral cytoplasm towards the end of the growth period. Such nuclei are found in the eggs of aculeate Hymenoptera, but do not occur generally in other groups; they have, however, been described by Hegner (1914) in the oocytes of the Braconid, *Apanteles.* Those in *Synergus* agree remarkably closely with the description given for the latter genus. Each has a definite membrane and contains a substance staining like chromatin in the form of several small masses and a few strands of granules. That they arise *de novo,* that is to say, they do not originate by the division of pre-existing nuclei, is agreed by Hegner as regards *Apanteles* and shown in *Synergus* by: (1) the absolutely constant form of the germinal vesicle at the time when they appear precludes any possibility that they arise by budding from it; (2) the complete isolation of the oocyte by its follicle at the time when secondary nuclei are first seen excludes any possibility that they are derived from nuclei other than those of the follicle cells; (3) careful examination of the follicle cells and their nuclei at this stage makes it extremely improbable that there is any migration of nuclei from the epithelium into the peripheral cytoplasm of the egg. Beyond this, Hegner makes no suggestions as to their presence; but it seems to me clear that in *Synergus* they arise from the groups of granules in the periphery that have been referred to above. The full story of their genesis would then be as follows: as the oocyte nucleus enters upon the diffuse stage its diminution of staining capacity is seen to synchronise with the ejection of chromatin-staining particles in the surrounding cytoplasm ; these particles increase in volume and subsequently undergo fragmentation, becoming limited by an enclosing membrane, and so simulating the appearance of true nuclei. Hegner states that secondary nuclei in *Apanteles* are only found for a brief period in the egg: this is probably the case in *Synergus* also.

4. *Orthopelma Luteolator.*

The later maturation prophases, as stated in the introduction to the present communication, have been described briefly by Hegner, Silvestri, Martin, and Gatenby in certain Chalcididæ and in the Braconid, *Apanteles*, I have examined various species of both families, including *Torymus*, *Microgaster* and *Hecabolus;* but while confirming in general their conclusions, have insufficient new matter to merit record. To emphasise, however, the general occurrence of the phenomena that have been described in *Cynips* and *Rhodites* among the parasitic Hymenoptera, it will suffice to give a brief survey of the history of the oocyte in a representative of a family upon which no cytological work has been performed up to date. *Orthopelma luteolator* is one of the Cryptine Ichneumonidæ, which parasitises the galls of *Rhodites* probably as a parasite on the gallmaker itself. A very large quantity of this species was bred in the course of my experiments with *Rhodites* itself; and I am indebted to Mr. Turner, of the British Museum, for assistance in the identification of this species.

In the larva, just before pupation begins, the ovaries consist of rosettes, the cells of which are undergoing segmentation by synchronous divisions. This is shown by the fact that mitoses are rare; but where they occur they are always seen in the same group of cells altogether (fig. 42). The number of chromosomes in dividing oogonia appears to be about 22. The eggs do not all develop at the same time, as in the Cynipidæ, this insect having a longer life in the adult condition. In imagines that have just emerged, synapsis can still be studied in the terminal portion of the egg-tubes, which are of the usual monothalamous type. Unfortunately, however, the nuclei are minute, the whole egg in *Orthopelma* being in size about a fifth that of *Rhodites.* No differential divisions either of the nucleus or cytoplasm occur in the rosettes, the cells of which are equipotential. There is no visible distinction between nurse cells and oocyte until after synizesis (figs. 43, 44). The ovaries of the adult contain oocytes in all stages of growth situated at the posterior end. Nuclei in synizesis may be found in the ovaries of the pupa, or of the early adults just after they make their way out of the gall. After the dissolution of the synaptic knot the differentiation of nurse cells and oocytes ensues immediately. The nurse cells in the region of differentiation possess nuclei with large plasmosomes and innumerable chromatic granules; but fragmentation is preceded by a stage in which the chromatin of the nuclei is distributed in the form of lumps of somewhat ragged outline (fig. 45). A large number of such nuclei were studied, and it seems that the number is constant and equal to half the number of chromosomes in the oogonia (10–12). I have been unable to satisfy myself that these chromatin masses show a division into two parts; but some sections suggest that this is the case, as in the young nurse cells of *Philosamia.*

The nucleus of the very young oocyte at the same stage shows what appears to be the haploid number of somewhat convoluted threads. At first I was of the opinion that there is no "diffuse" stage in the oocyte nucleus of *Orthopelma;* and Hegner omits any mention of one in the case of the Chalcid *Copidosoma,* or the more closely allied form *Apanteles.* This is comprehensible on account of the fact that owing to the rapid development of the oocyte, it is impossible to obtain a complete sequence of stages without the examination of a large number of preparations; and it is easy to attribute the disappearance of individually recognisable chromosomes to defective preservation. After comparing preparations of the ovaries from some fifty females, I am convinced that such a stage does occur, and that it is followed by the reappearance of the diploid number as in *Copidosoma* followed by an end-to-end pairing of univalent elements (figs. 47 and 48). I am convinced equally that if the case of *Copidosoma* or *Apanteles* were reinvestigated from this point of view a "diffuse" stage would be found to follow synapsis in these genera likewise. An abortive maturation spindle is formed by the arrangement of these pairs (eleven in number) on an asterless spindle (fig. 49). The bivalent chromosomes of the spindle are short stout rods as in *Rhodites;* in none of the forms examined either by Gatenby, Martin, or myself have the chromosomes been found to exhibit the filamentous form figured by Hegner, whose illustrations are evidently somewhat diagrammatic. As in all these cases the chromosomes condense, and the spindle fibres eventually disappear as the nucleoplasmic zone surrounding the chromatin complex is invaded by the yolk. In other forms I have examined the condensation figure is of homogeneous consistency; but in *Orthopelma* as in *Andricus* (Hegner) it exhibited a vacuolated structure (fig. 51).

Chromatin-staining granules occur in the young oocyte, but no secondary nuclei. In the full-grown egg there is a triangular area of minute granules at the posterior pole which condense as in *Apanteles* to form a polar disc or oosoma of crescentic shape (fig. 52).

The foregoing account of the oogenesis of an Ichneumonid confirms the view that a remarkable similarity exists in the behaviour of the nucleus of the oocyte throughout the large families of the parasitic Hymenoptera (Chalcididæ, Braconidæ, Cynipidæ, and Ichneumonidæ). There can be little doubt that the phenomena described are general within the group and contrast strongly with the oogenesis of other Insecta and the remaining families of Hymenoptera. The most noteworthy features on which attention has been focussed are the peculiar interruption of the first polar mitosis and the existence of a second syndesis immediately before the formation of the maturation spindle.

5. *Lasius Flava.*

In order to contrast the peculiar phenomena which have been described in the oogenesis of the parasitic families of Hymenoptera, it is proposed to complete this account with a brief survey of the history of the egg nucleus in the yellow ant, *Lasius flava*, as exemplifying the Aculeata.

Though the maturation of the laid egg and the spermatogenesis of various Aculeata have been somewhat extensively studied, the synaptic phase has been overlooked by most authors, or at least treated very imperfectly. Paulcke (1900) made a study of nuclear differentiation in the ovarioles of *Apis*, and came to the conclusion that all the cell elements are germinal in origin, finding no visible distinction between oocytes and nurse cells until the zone of synapsis is passed. Nachtsheim's figures are similar.

Towards the end of pupal life in *Lasius* all stages of oogenesis can be found in the ovarioles, except the full-grown egg. The terminal filaments consist of flattened cells with granular nuclei, arranged in horizontal rows and extending across the entire width of the tube. At the point of origin of the "terminal chamber" mitoses are frequent. In a small number of very clear figures, 24 minute chromosomes were counted. The nuclei of the terminal chamber, as in *Campanotus*, are not limited by cell walls, but from a syncytium. In the chamber are found successively (*a*) rosettes; (*b*) a zone of synapsis; (*c*) a zone of differentiation, where nurse cells and oocytes become recognisably distinct. At first the nurse cells and oocytes are indiscriminately scattered. In the lower part of the ovariole they are arranged in the characteristic manner, the oocytes forming an axial series alternating with nurse chambers and surrounded by a definite follicular epithelium (fig. 57).

The large number and small size of the chromosomes in the Hymenoptera generally render the group most unfavourable for a crucial elucidation of the synaptic phase, but in studying the ovarioles of *Lasius*, the more satisfactory seriation of stages permitted a better perspective with respect to those changes which occur immediately before the formation of the synaptic knot than forms previously discussed.

The preleptotene nuclei of *Lasius* exhibit a large plasmosome and the characteristic clumps of chromatin present in insect gametocytes at this stage, and it is possible to trace their transition into the paler and convoluted tangle of leptotene filaments in the following stage (fig. 53). In *Lasius* the leptotene threads definitely arrange themselves in such a manner as to converge towards one pole of the nucleus before passing into synizesis (fig. 53); further, it can be definitely stated that in some "bouquet" nuclei there are a large number of pale threads, and in others a much smaller number of thicker and more deeply staining filaments (fig. 53), indicating a possible condition of pachynema.

Synizesis is illustrated in fig. 54. Till this stage no distinction can be recognised in the cells of the terminal chamber. The haploid number of filaments appear after the break up of synizesis. Differentiation follows with remarkable alacrity. Already among the cells showing nuclei with separate pachytene filaments (after synizesis) two types can be distinguished. In the future oocytes the surrounding cytoplasm is already more bulky and granular. In the case of oocytes a diplotene stage is intercalated (fig. 56). What follows in each case is in the main similar to the course of events in the forms treated earlier in this paper. The chromatin of the nurse cell nuclei undergoes fragmentation into minute granules: at a very early stage the large and characteristic nucleolus can be seen. The oocyte nucleus shows no plasmosome; the filaments suffer a great diminution of staining capacity and lose their visible identity. There is contemporaneously a reduction of the chromatin-content of the nucleus. In the early stages of growth the nucleoli of the nurse cells constantly undergo fragmentation and eject particles, which can be seen to pass into the protoplasmic strand connecting the young oocyte with the nurse chamber.

The Late Ovarian Oocyte.

The nucleus remains in this condition until about the time the egg is laid, when various authors have described a typical spindle. None of the peculiar phases that are intercalated in the late ovarian oocyte of parasitic species like *Cynips* or *Copidosoma* occur. Since in both the ant and the bee the first polar spindle is already formed when the egg is laid the late maturation prophases must be studied immediately before the laying of the egg. With this end in view I have examined the oldest eggs in young queens of *Lasius* without success and preparations from *Formica rufa* of the ovarioles of queens taken after the marriage flight from nests in which eggs were being laid, yielded equally fruitless results. There is, however, little reason to doubt that they are of the kind characteristic of insect oogenesis.

6. *Secondary Nuclei of Formica rufa.*

Secondary nuclei were first described in the oocyte of the Formicidæ by Blochmann who also described the presence of symbiotic bacteria in the eggs of some species. To the former Blochmann applied the term "Nebenkern." But as various cell structures, including the spindle apparatus, mitosome and the Golgi rods, have been by different authors described by the same word, it is entirely misleading to designate them thus. Their presence has been confirmed by several authors both in the ants, wasps and bees; but their origin and fate has proved fruitful of much controversy. Marshall and Blochmann both believed that they arise by budding from the germinal vesicle, but no actual stages in the budding were observed, and in view of the extreme improbability of amitotic division in germ cells, it is gratuitous to base such a conclusion on the such slender data as their first appearance in the neighbourhood of the oocyte nucleus and their irregular shape. Henneguy and Brunelli believe that they are derived from the follicular epithelium. Korschelt and Gross conclude the same in the case of *Bombus*, and Will (1884) finds that such bodies when they occur in the Hemiptera have a similar history.

The most thorough studies on the subject are those of Loyez who describes "pseudo-noyaux" in four species of *Bombus*, two of *Vespa* and in *Xylocopa*, and concludes that they originate from granules ejected from the nucleus of the oocyte, nurse cells and oocyte follicles, transformed into nucleoform bodies by the cytoplasm of the egg. This is what I believe to occur in *Synergus*, loss of nuclear matter in resting nuclei and its ejection as "chromidia" into the surrounding cytoplasm occurs in many insects, and clearly in the growth period form the germinal vesicle of *Formica rufa.*

Such secondary nuclei have been described in two species of *Campanotus* and in *Formica fusca* in the case of the ants. In the former they appear very early—before the egg is separately surrounded by a follicle, and as Hegner points out this is a potent objection to the theory that they arise by the migration of complete nuclei from the follicular epithelium. From my own preparations I cannot say that they appear at such an early date in *Formica rufa*, but the enormous number that are found in the full grown egg would inevitably be recognisable from the presence of follicular nuclei in the process of division were this hypothesis acceptable. Actually, however, follicular mitoses are not more numerous than would be expected, and no evidence of indirect nuclear division or migration was observed. In the earliest stages on the contrary the secondary nuclei were always seen in the immediate neighbourhood of the oocyte nucleus. Further it may be added that no intermediate conditions were seen between the nuclei of the follicular cells and of the oocyte which are manifestly different. The nuclei of the follicles are of the characteristic granular type (fig. 60*a*), while those of the secondary nuclei (fig. 60*b*) were distinguished by chromatin-like masses often radially disposed and sometimes clumped together in a single mass (fig. 60*b*).

Regarding the subsequent fate of these structures there is insufficient ground for a final judgment in my own studies on *Formica rufa*, but on the whole I can confirm Loyez' view. The secondary nuclei in *Apanteles*, *Synergus* and *Rhodites ignota* appear to be temporary bodies which disappear without leaving any trace of their identity. According to Loyez those of *Bombus*, *Vespa* and *Xylocopa* are ultimately transformed into deutoplasmic spheres. Marshall believes that they assist the cytoplasm in the anabolic processes involved in the assimilation of material from the yolk discs. Hegner ('09), who has availed himself of every suggestion in favour of the nuclear origin of the "germ-cell determinants," has expressed the belief that they may migrate to the posterior pole, and take part in the formation of the latter; but no actual evidence either that they do migrate or have any connection with the "germ-cell determinants" is submitted. I do not believe that there is a "polar disc" or oosoma in *Formica rufa*, nor have I found any evidence of the migration of secondary nuclei to the posterior pole of the egg, so that the last hypothesis may be rejected. That of Marshall is suggestive and not improbable; but it seems to exceed the legitimate limits of inference on the data available. Loyez claims that all stages between the typical secondary nuclei described above and older nuclei simulating the condition of synizesis and homogeneous globules can be observed.

In favour of this view, *Formica rufa* supplies the following data: (i) towards the end of the growth period and immediately before the first appearance of yolk spheres, the secondary nuclei cease to form a compact group round the germinal vesicle and are distributed throughout the egg, particularly in the periphery; (ii) yolk deposition proceeds from the periphery inwards; and the yolk discs are large bodies of dimensions comparable to those of the "pseudo-noyaux; (iii) in my own preparations, the later secondary nuclei are seen in a condition simulating synizesis. On the whole, therefore, there is some degree of probability in favour of the view that the secondary nuclei of the aculeate Hymenoptera are destined to become transformed into deutoplasmic spheres; that they are connected in any way with the "polar disc" seems both improbable and unacceptable.

Synopsis.

(1) The chromosome cycle has been investigated in relation to the production of females from virgin eggs in the Hymenoptera ; the process of synapsis and the maturation of prophases of certain parasitic Hymenoptera have been studied and contrasted with similar stages in the Aculeata; the origin and fate of secondary nuclei has also been considered.

(2) With respect to three genera of parasitic Hymenoptera, the origin, differentiation, and nuclear history of the oocytes have been investigated, viz., *Cynips* and *Rhodites* (Cynipidæ) and *Orthopelma* (Ichneumonidæ). The results confirm the existence of a somewhat unique type of oogenesis, as suggested by available particulars relating to Braconid, Chalcid, and Cynipid genera described by Hegner and other authors. The more salient points are:—

(a) *Origin of the Oocytes.*—All three types of cells (oocytes, follicle, and nurse cells) in the ovary originate from germ cells. The divisions of the oogonia are apparently equipotential, and no distinction exists between nurse cells and oocytes until after synapsis.

(b) *Synapsis*—After synizesis the haploid number of pachytene threads appear, and in *Rhodites* split lengthwise.

(c) *The "Diffuse" Stage.*—During the growth period there is a diffuse or confused stage.

(d) *The Double Conjugation of the Chromosomes.*—At the termination of the growth period the diploid number of chromosomes reappears, and they pair end to end, as already described by Hegner in the Chalcid *Copidosoma*, so that, as in *Lepidosiren*, a temporary separation of the diplotene threads is intercalated between synapsis and the first polar mitosis. *This is the first confirmation of Agar's account of a double syndesis yet published. The restoration of the diploid number by the lengthwise splitting of the haploid pachytene threads permits the inference that the first conjugation is of the type described as "parasyndesis."*

(e) *The Abortive Maturation Spindle.*—Immediately after this the maturation spindle appears precociously in the late ovarian oocyte; it shows no asters or centrosomes. The chromosomes on the equatorial plate condense to form a solid "chromatin nucleus" without proceeding to the poles of the spindle, which eventually disappear. Such behaviour is without a parallel in the ookinesis of other forms previously investigated. In all genera where such abortive spindles have been shown to occur, the separation of the first group of polar chromosomes is atypical. There can be little doubt that we have here an interrupted mitotic process.

(3) *Agamic Female Production.*—With regard to the production of females from parthenogenetic eggs, the case of *Rhodites* and *Cynips* has been considered, and Henking's account of the former has been vindicated. *It appears that there is a reduction of the number of chromosomes in the young oocyte of both these forms, and it may be assumed that Henking rightly described the double-equational formation of polar bodies with subsequent disjunction of univalent halves in the female pronucleus of the former.* This is not in conflict with the case of *Neuroterus*, where, although no polar bodies are formed in the female-producing ova of the agamic generation, all the oocytes appear to show the reduced number of chromosomes at the corresponding stage.

(4) A study of the early oogenesis of *Lasius* demonstrated that synizesis is followed by the appearance of the haploid number of chromatin threads, and the differentiation of nurse cells from functional oocytes; and the oocyte nucleus remains in the "confused" stage till immediately before it is laid.

(5) *The Secondary Nuclei.*—Secondary nuclei have been described in *Synergus* (Cynipid) and *Formica rufa*, and their genesis has been examined; my conclusions with regard to their origin and fate are, that in both the case of *Synergus* and *Formica*, they arise from nuclear particles, the ejection of which synchronises with a diminution of staining capacity in the germinal vesicle. These migrate to the cytoplasm, fragment, and acquire an enclosing membrane; they are transitory structures. Similar conclusions have been recorded by Hegner in regard to *Apanteles* and *Rhodites ignota.*

(6) *The Oosoma.*—The Oosoma, or so-called germ-cell determinant, is not of nuclear origin, as believed formerly by Hegner and Silvestri, but arises, as Gatenby affirms, from cytoplasmic granules which are not mitochondrial.

The present research was commenced at Birkbeck College. The greater part has been conducted at the Imperial College. I must express my thanks to Prof. MacBride, who has kindly read through the MS., and assisted me with helpful criticism. To Prof. Doncaster, of Liverpool—whose kindness in sending material of *Neuroterus* for a previous paper first directed my attention to the cytological interest of the Hymenoptera—I owe special gratitude, for continuous help with regard to bibliography, and suggestive advice throughout.

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EXPLANATION OF PLATES.

Illustrations were outlined with camera lucida and studied with Leitz 2 mm. and No. 12 apochrom. Single cells of individual plates are drawn at the same magnification. Figs. 9*b*, 10, 30, 31, 39, 40, 41, 46, 52, are not drawn at the same magnification.

Plate 4.—*Cynips Kollari.*

Fig. 1, *a*, *b*, *c*,—*Cynips Kollari* side views of mitoses in somatic tissues.
d, *e*.—Anaphase plates from gut of larva: 20 chromosomes visible.
Fig. 2.—Follicular mitoses with 20 chromosomes.
Fig. 3.—Cell from ovary of larva during resting period.
Fig. 4.—Rosette cell, showing irregular clumps of chromatin in the nucleus.
Fig. 5.—Leptonema. Dense tangle of pale threads in the nucleus.
Fig. 6, *a*, *b*, *c*.—Stages in synizesis.
Fig. 7, *b*, *c*.—Young nurse cells.
Fig. 8.—Young oocyte showing pachytene threads haploid number.

Plate 5.—*Cynips Kollari* (continued).

Fig. 9, *a*.—Young oocyte, "diffuse stage." Chromosomes with reduced staining capacity, identity no longer traceable.
b.—Somewhat later oocyte less magnified, showing follicle nurse cells and protoplasmic strand of egg protruding into the nurse chamber.
Fig. 10.—Diagrammatic representation of the full-grown egg.
Fig. 11.—Nucleus of oocyte at end of the growth period. Diploid number of filaments visible.
Fig. 12.—End to end pairing of the same.
Figs. 13, 14.—Maturation spindle of the late ovarian oocyte with 10 doubles.
Figs. 15–17.—Stages in condensation of the abortive maturation spindle: disappearance of spindle fibres and reduction of the nucleoplasmic zone.

Plate 6.—*Rhodites Rosæ.*

Fig. 18.—Follicular mitoses.
Fig. 19.—Oogonial mitoses showing 18 chromosomes.
Fig. 20.—Rosette from ovary of late larva with chromatin of nucleus in form of irregular masses.
Figs. 21, 22.—Leptotene nuclei.
Figs. 23–25.—Synizesis figures.
Figs. 26, 27.—Post-synaptic spireme segmenting into 9 pachytene threads.
Fig. 28.—Diplotene nucleus.
Fig. 29.—Late diplotene in a young nurse cell comparable to that described by Dederer in Philosamia.

Plate 7.

Fig. 30.—Very young oocyte and adjacent nurse chamber. The oocyte nucleus is in the diffuse stage, with chromatin granules in the surrounding cytoplasm. The nuclei of the nurse cells are granular. (Magnification reduced.)

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BIOLOGICAL SCIENCES.

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Fig. 31.—Full-grown egg (diagrammatic), showing deeply staining body (polar disc) at posterior pole and nucleus at the periphery at one side.
Fig. 32.—Nucleus after the "diffuse" stage with diploid chromosomes: vacuolated plasmosome and two karyosomes.
Fig. 33.—End to end pairing of filamentous chromosomes.
Fig. 34, *a*, *b*, *c*.—Showing haploid number of bivalents with condition indicating their double nature; ring-like karyosomes and plasmosomes.
Fig. 35.—Formation of maturation spindle.
Fig. 36.—Abortive maturation spindle.
Figs. 37, 38.—Condensation of same.

PLATE 8.—*Synergus and Orthopelma.*

Figs. 39–41.—*Synergus Rheinhardii.* Formation of Oosoma and secondary nuclei.
Fig. 39.—Cloud of granules at posterior end: spherical chromatin granules disposed in the cytoplasm.
Fig. 40.—Granules at posterior pole condensing. Large chromatin granules fragmenting in the periphery of the egg.
Fig. 41.—Secondary nuclei in periphery of egg; *g.v.* = germinal vesicle.
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Fig. 45.—Young nurse cell: haploid number.
Fig. 46.—Young oocyte and nurse chamber. Nucleus shortly before "diffuse" stage shows haploid number of bivalents. (Magnification reduced.)
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Fig. 50.—Condensation of same.
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PLATE 9.—*Lasius and Formica.*

Figs. 53–57.—*Lasius flava.*
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Fig. 55.—Haploid number of pachytene threads from post-synaptic spireme.
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Figs. 58–60.—*Formica rufa.*
Fig. 58.—Young oocyte with follicle complete: secondary nuclei around germinal vesicle.
Fig. 59.—Later: migration of secondary nuclei to periphery.
Fig. 60*a*.—Follicle cell and nucleus.
Fig. 60*b*.—Yolk and secondary nuclei of full-grown egg.

Q B 840 (1920)

The Enzymes of B. Coli Communis *which are Concerned in the Decomposition of Glucose and Mannitol.* Part IV.—*The Fermentation of Glucose in the Presence of Formic Acid.*

By Egerton Charles Grey (John Foulerton Student).

(From the Biochemical Laboratory, Cambridge.)

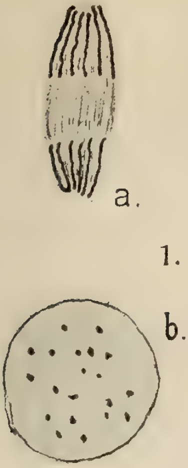
(Communicated by F. Gowland Hopkins, F.R.S.—Received May 3, 1920.)

In this series of communications the writer is endeavouring to show how, by varying the conditions of the experiment, it is possible to alter the proportion between the products which arise from the fermentation of glucose and allied substances, and to point out how, by a consideration of the manner in which these products group themselves, conclusions may be drawn as to the order in which such products arise during the degradation of the glucose molecule. Substances which can be shown to arise in constant proportions under varying conditions of experiment may be considered as being produced by one and the same enzyme.

In Parts II and III it was shown that the formation of lactic acid by *B. coli communis* ran a separate course to that of the other products, so that it may be regarded as being produced by a separate enzyme, but the other products of the fermentation, viz., succinic acid, acetic acid, formic acid, and alcohol, together with the gaseous products of the decomposition of formic acid, *i.e.*, carbon dioxide and hydrogen, all appeared to be grouped together and to form an alternative course for the decomposition of the glucose.

In the present communication it will be shown that this group of products must be subdivided into two groups, the one containing formic acid and its gaseous products carbon dioxide and hydrogen, and the other alcohol, succinic acid, and acetic acid.

The possibility of demonstrating the independence of formic acid on the one hand and acetic acid, alcohol, and succinic acid on the other, depends on finding a means of destroying the normal balance between these products. It is probable that this balance is conditioned by the hydrogen which arises through the decomposition of the formic acid, for this hydrogen might reduce acetaldehyde to alcohol, and in this way the formation of formic acid and alcohol would tend to keep pace with each other. However this may be, it is not difficult to upset this balance, and one method of so doing is to ferment the glucose in the presence of added formic acid in the form of calcium formate. How the added formate is able to upset the normal balance which exists between the products of this group, the writer is not prepared to say.



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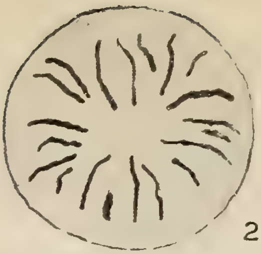

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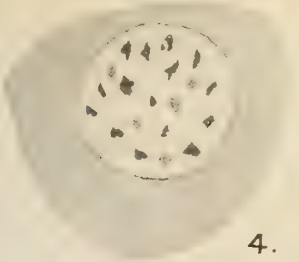

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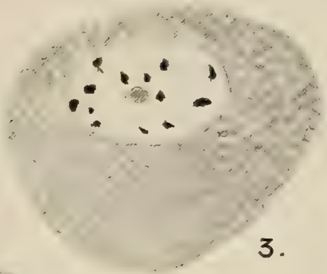

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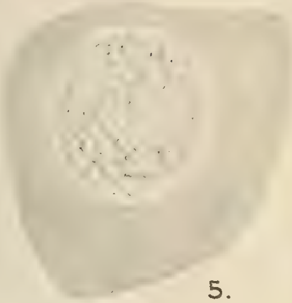

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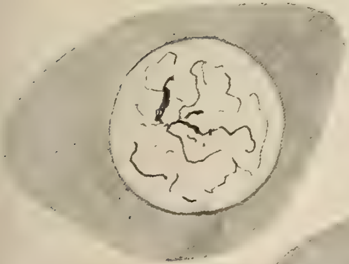

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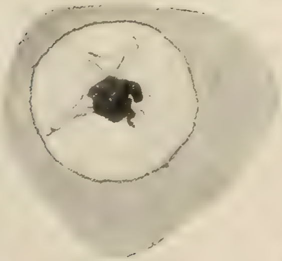

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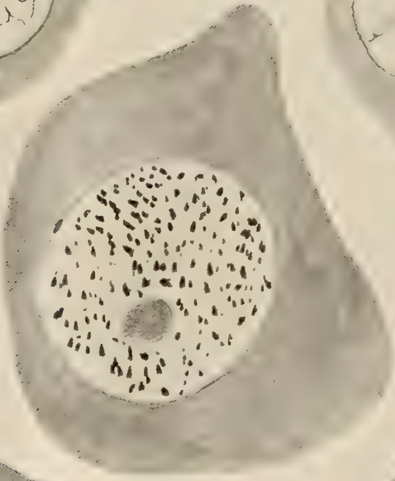

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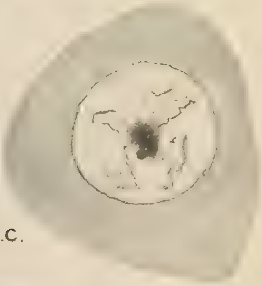

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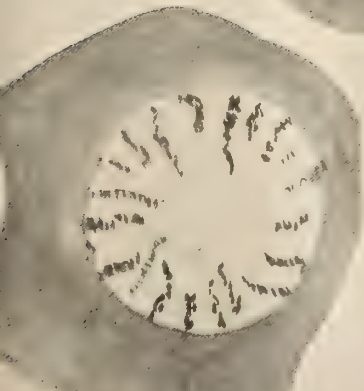

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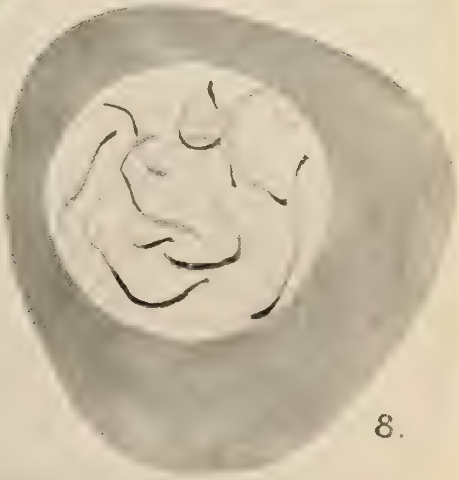

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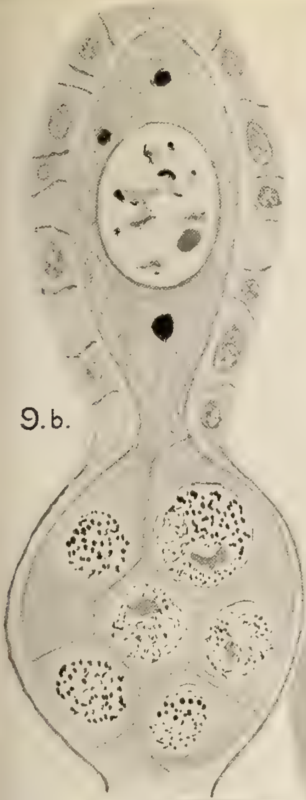

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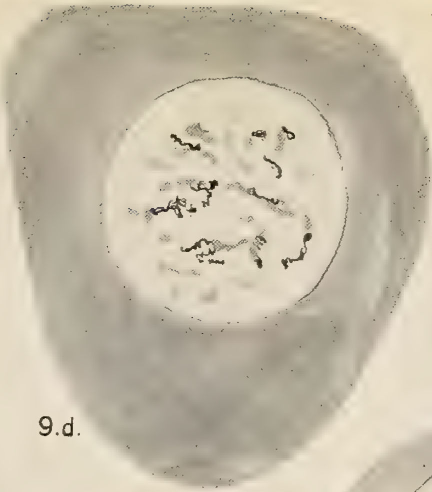

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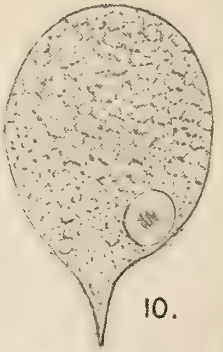

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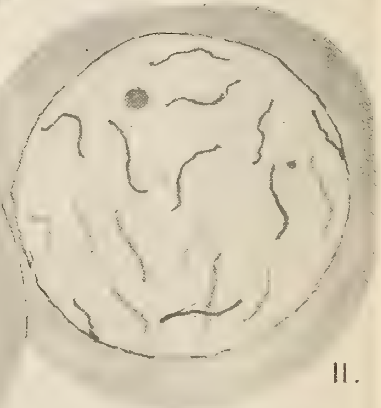

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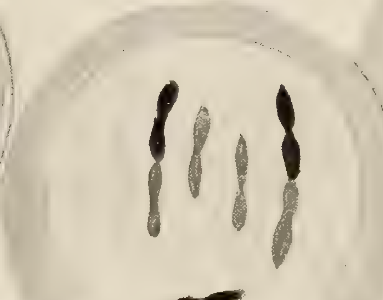

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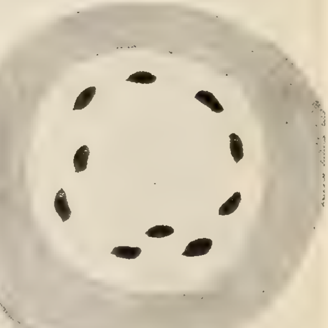

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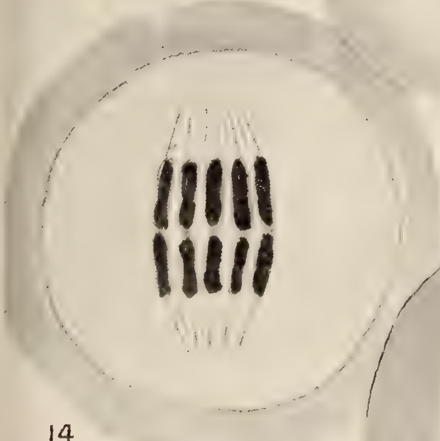

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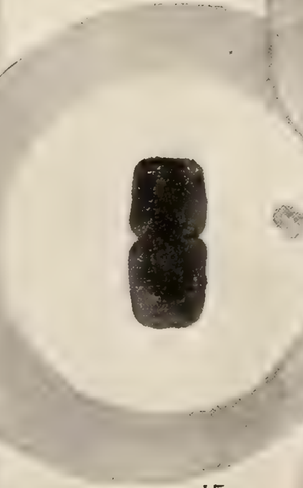

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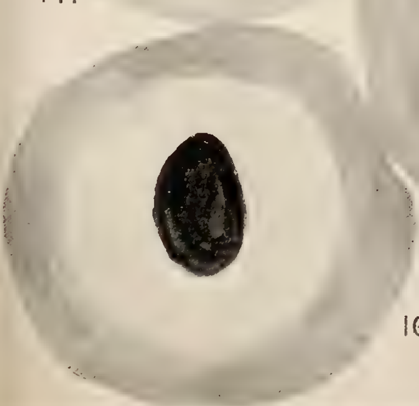

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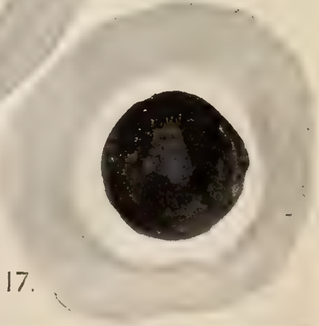

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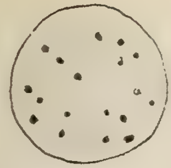

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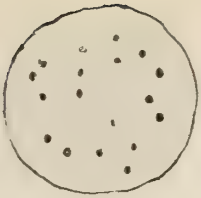

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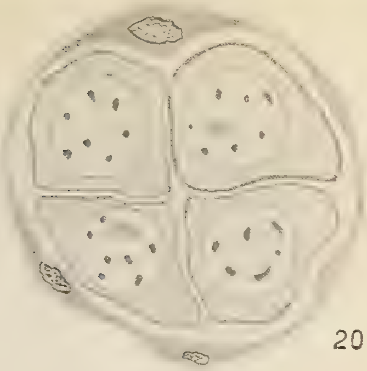

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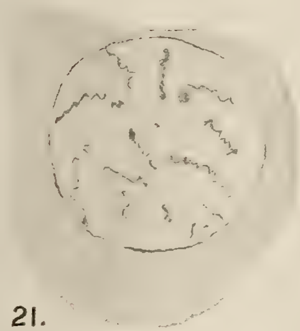

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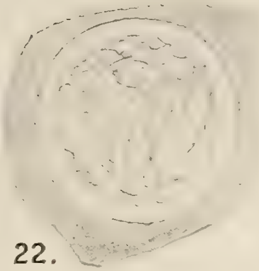

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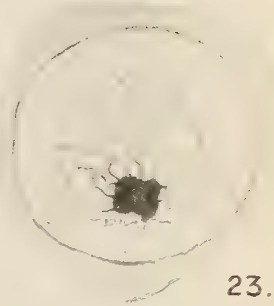

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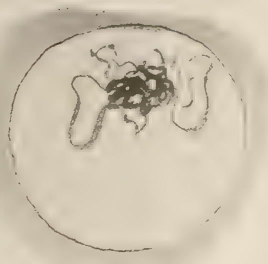

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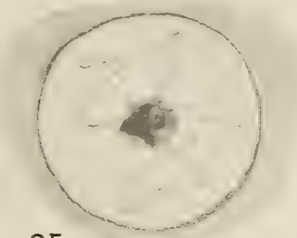

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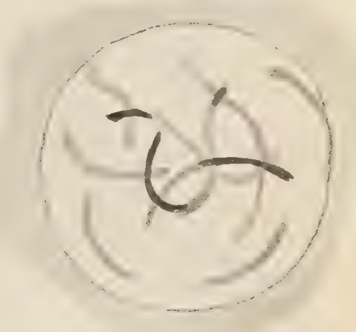

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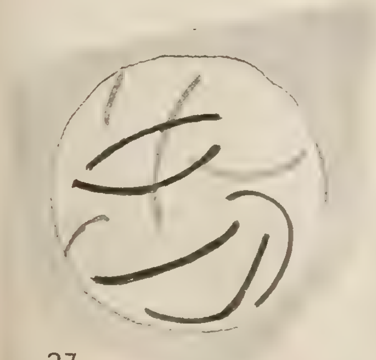

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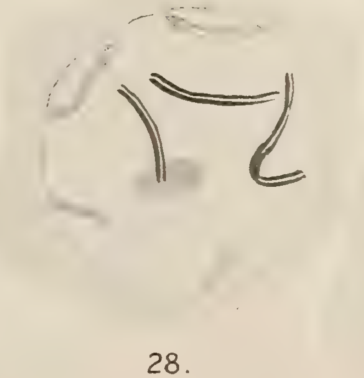

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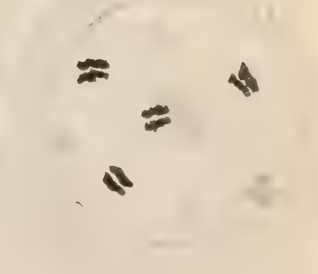

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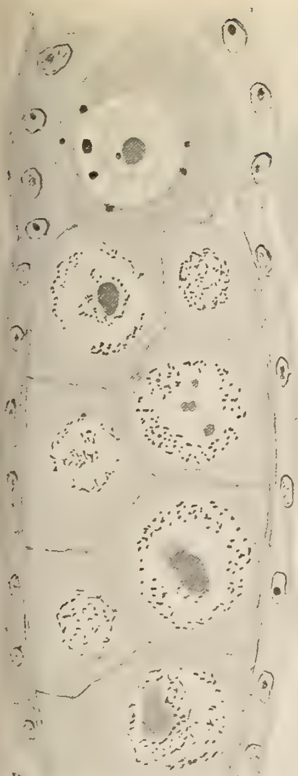

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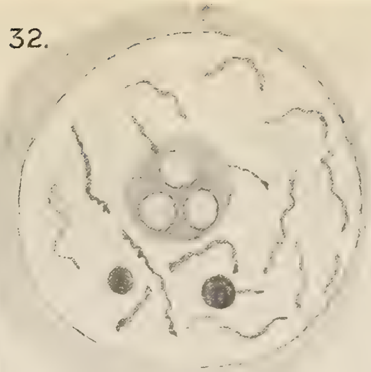

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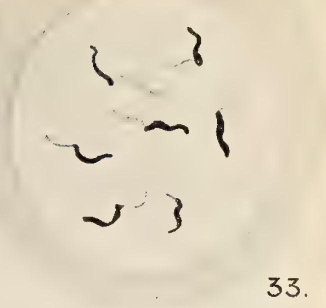

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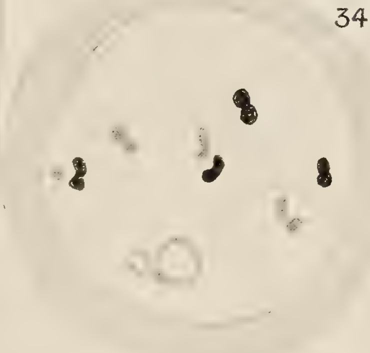

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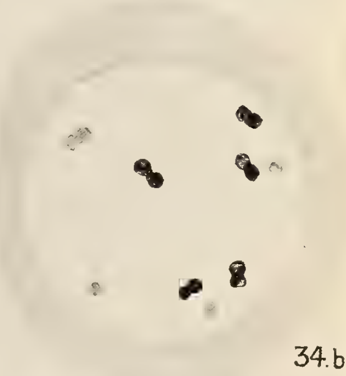

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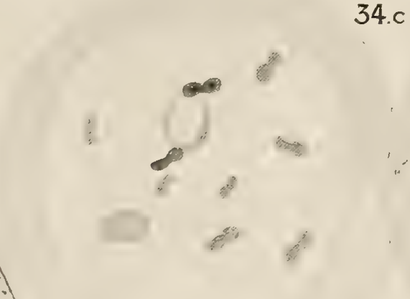

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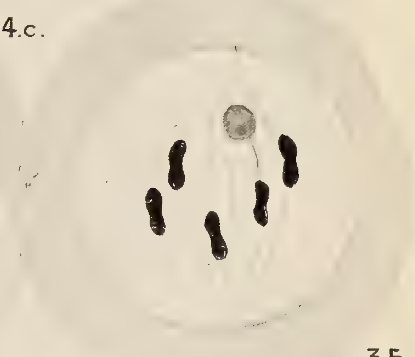

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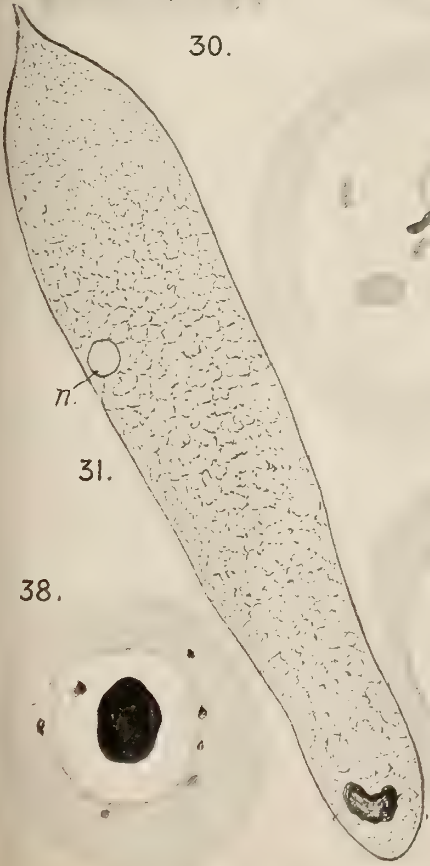

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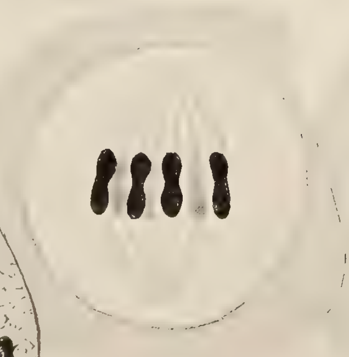

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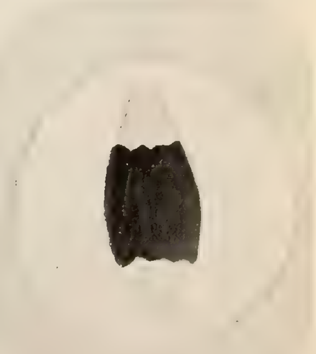

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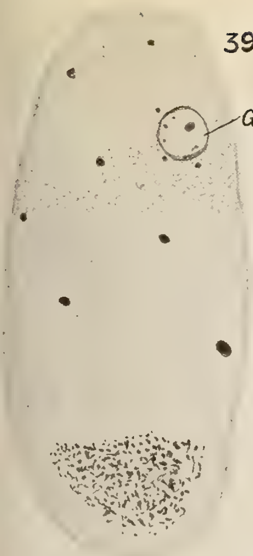

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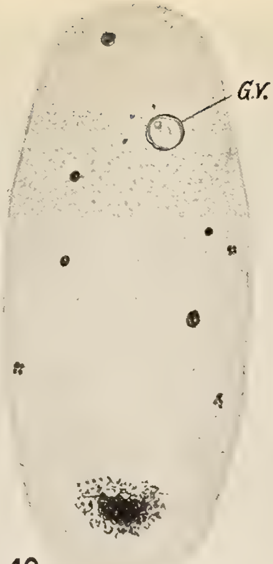

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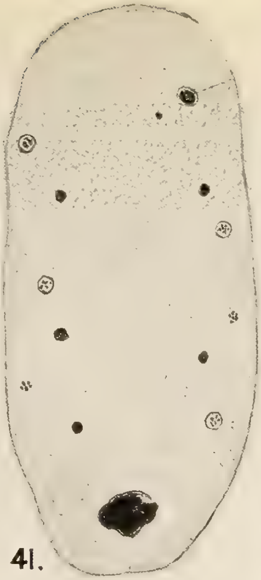

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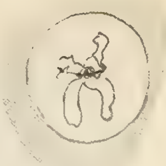

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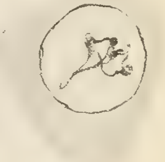

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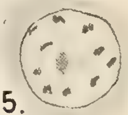

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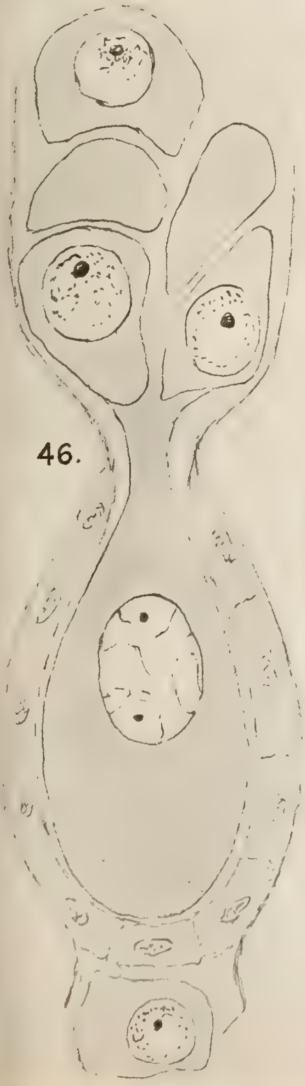

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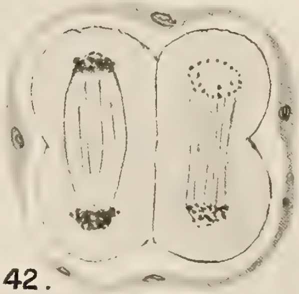

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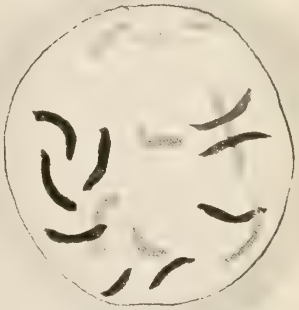

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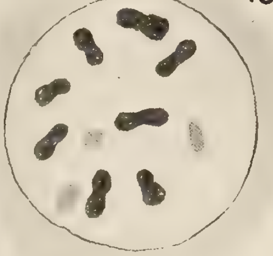

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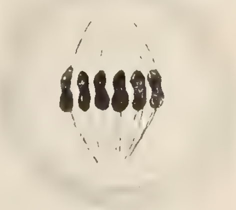

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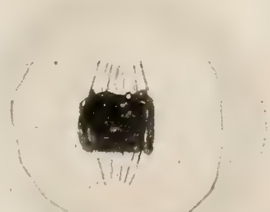

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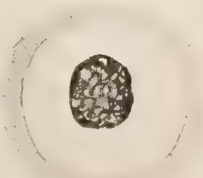

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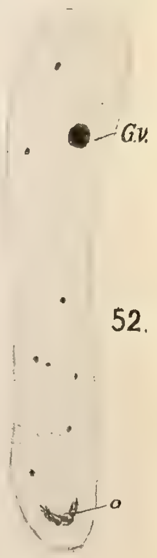

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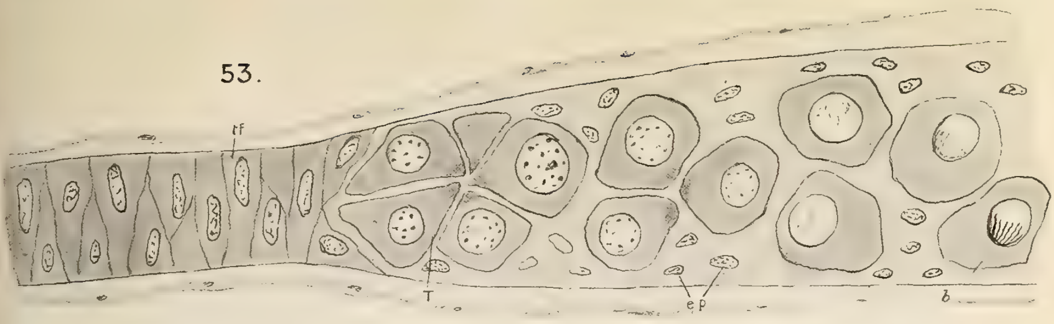

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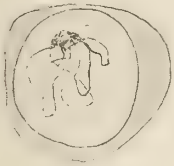

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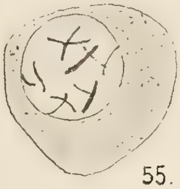

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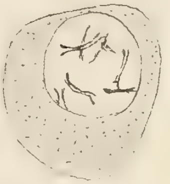

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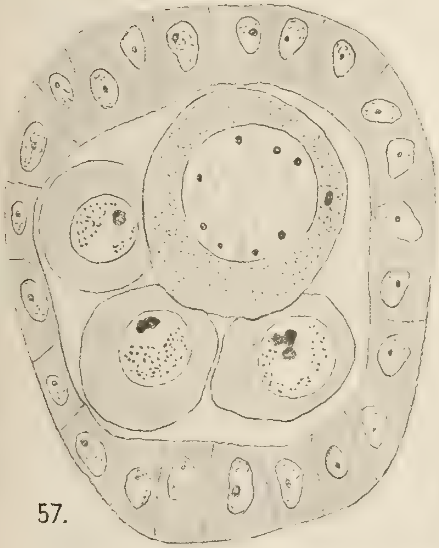

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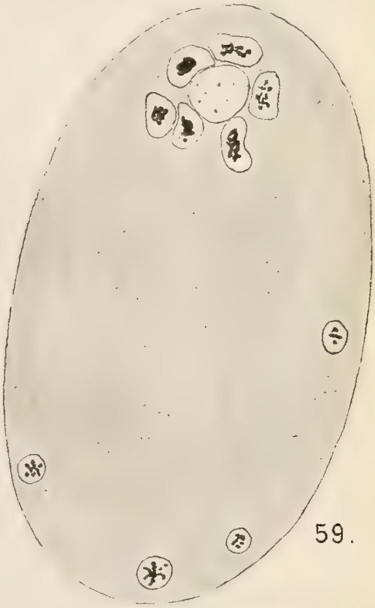

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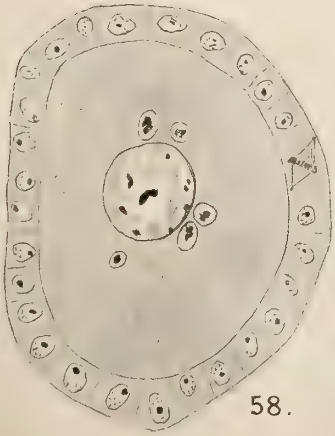

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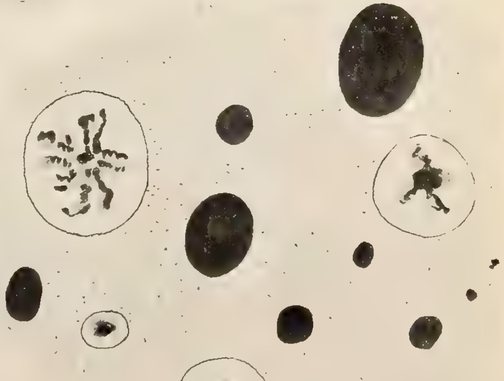

The present experiments were undertaken rather with the object of seeing whether the hydrogen nascent during the decomposition of formates could give rise to an increased yield of alcohol from the glucose. In one case such an increase actually occurred, but in most cases the reverse was the case. On the other hand, the results have been valuable in themselves for the reason stated above.

It must be confessed that the concentrations of calcium formate employed in these experiments appear to have been chosen somewhat at random. One of the reasons for this is the difficulty of deciding upon the use of varying concentrations of calcium formate, and at the same time keeping the concentration of salts within proper bounds. The criticism which may be raised on these grounds in regard to the order in which these experiments were carried out does not, however, in any way concern the conclusions which are drawn in the present communication. The results are considered alone in their relation to one another and not in regard to the cause, whatever it may be, which is responsible for them.

Outline of the Method Employed.

An emulsion of *B. coli communis* was prepared by the method described in Part II, and a quantity representing about 0·5 grm. of dried bacteria was added to a solution previously sterilised by heat, containing either glucose, or glucose together with calcium formate. The solution also contained potassium sulphate 0·6 per cent., and magnesium sulphate 0·1 per cent. as in Part III. Chalk was added to neutralise the acid which might arise during the fermentation.

In order to know the proportion of the products which arise from the glucose, it is necessary to subtract from the total products those which arise from the decomposition of the added formate. Accordingly it is necessary to determine at the end of each experiment how much formic acid remains, and to deduct from the gaseous products an amount of hydrogen and carbon dioxide equivalent to the formic acid which has been fermented.

In most cases the amount of formic acid present at the end of the experiment was less than at the start although, considering the large amount of gases evolved, it was surprising to find that the actual diminution in formic acid was not great. The formic acid was never exhausted, and in one case there was an actual increase in the formic acid over that present at the start.

The data for a series of experiments are given in Tables I and II.

Table I.

Data for experiments on the decomposition of glucose and mixtures of glucose and calcium formate, by *B. coli communis.*

	0.	1.	2.	3.	4.
Weight of glucose at the start	20·00	20·72	20·00	19·68	16·25
Weight of formic acid	1·320	1·325	3·312	3·750	5·244
Weight of glucose at the end	4·73	7·95	3·13	4·76	Nil
Weight of formic acid	0·4233	1·708	2·282	1·380	4·269
Weight of formic acid fermented	—	—	1·030	2·370	0·975
Weight of glucose fermented	15·27	12·77	16·87	14·92	16·52
Weight of hydrogen produced	0·0495	0·1530	0·3234	0·2250	0·1260
Weight of hydrogen corresponding to added formate fermented	—	—	0·0453	0·1017	0·0417
Weight of hydrogen derived from the glucose fermented	0·0495	0·1530	0·2781	0·1233	0·0843
Weight of carbon dioxide evolved	5·901	7·003	9·953	8·448	4·950
Weight of carbon dioxide corresponding to the added formate fermented	—	—	0·986	2·266	0·933
Carbon dioxide from acids on chalk	3·495	3·080	2·160	0·726	1·243
Carbon dioxide from the glucose	2·497	3·922	6·807	5·460	2·770

Table II.

Products derived from glucose by the action of *B. coli communis* in the presence of calcium formate. Allowance has been made for the carbon dioxide and hydrogen which have resulted from the decomposition of the calcium formate added.

	Products from the glucose.				
	Glucose alone.	Glucose with calcium formate.			
	0.	1.	2.	3.	4.
Hydrogen	0·050	0·153	0·278	0·123	0·084
Carbon dioxide	2·497	3·922	6·807	5·460	2·770
Formic acid	0·422	0·380	—	—	—
Acetic acid	2·952	2·400	4·212	2·149	2·505
Lactic acid	4·347	4·030	3·654	2·770	8·533
Succinic acid	3·103	1·534	0·549	1·770	1·823
Alcohol	1·686	0·640	0·244	2·560	0·466
Total products	15·06	13·06	15·74	14·83	16·18
Glucose fermented	15·27	12·77	16·87	14·92	16·52
Ratio CO_2/H_2	2·04	1·17	1·11	2·01	1·50

In Table III the above results are calculated as percentages upon the sugar fermented.

Table III.

Products of the action of *B. coli communis* on glucose, calculated as percentages upon the glucose fermented. In the cases where the fermentation was carried out in the presence of calcium formate, allowance has been made for the hydrogen or carbon dioxide which has arisen from this source, so that the results tabulated represent products from the glucose itself.

	Products as percentages upon the glucose fermented.				
	Glucose alone.	Glucose fermented with calcium formate.			
	0.	1.	2.	3.	4.
Hydrogen	0·33	1·20	1·64	0·82	0·51
Carbon dioxide	16·37	30·71	45·41	36·60	16·77
Formic acid	2·76	2·98	—	—	—
Acetic acid	19·34	18·79	24·97	14·41	15·17
Lactic acid	28·47	31·56	21·61	18·57	51·65
Succinic acid	20·32	12·01	3·26	11·87	11·04
Alcohol	11·04	5·01	1·45	17·16	2·82
Total products	98·63	102·26	98·34	99·43	97·96

It will probably be admitted that these results are surprisingly accurate. Many analyses have to be carried out to obtain the proportions of all the products of such fermentations as will be seen for some of the data in Table I, and it may be fairly claimed that the present results are very satisfactory, and form a good basis for a consideration of the relations between the products concerned. It is essential to insist upon this fact, because, in the writer's opinion, the results recorded here are of fundamental significance in regard to the whole question of the manner of decomposition of the glucose molecule under the influence of bacteria. Such generalisations will, however, be reserved till Part VI of this series of communications, and in the present only the most obvious conclusions will be drawn.

It will assist in the consideration of these results to adopt the practice of Parts III and IV, and record the percentages of products in the form of curves. Such curves are obtained by plotting the percentages of the various products in columns corresponding to the individual experiments, the columns being arranged from left to right in order of ascending values for some one product. Since lactic acid has been shown to be formed independently of the other products, it is convenient to arrange the columns in order of increasing values for this substance. This has been done in fig. 1.

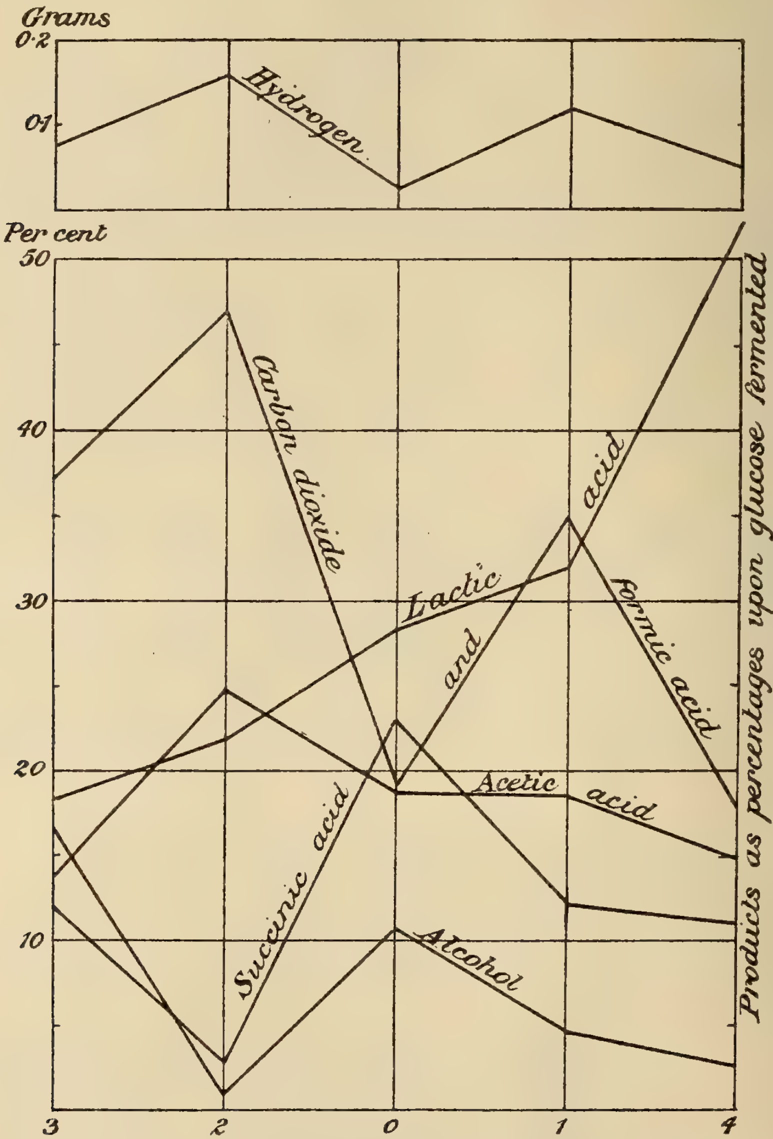

Fig. 1.—(Results arranged in order of increasing values of lactic acid.)

If fig. 1 is compared with figs. 2 and 3 of Part II of this series,* it will be seen that there is a very general resemblance between the curves, and especially in the respect that they all show the formation of lactic acid to be independent of the formation of the other products. On the other hand, there is a greater divergence of the curve for formic acid and its gaseous products from the other curves in fig. 1 of this present communication than there is in any of the previous figures given. It will also be noticed that the curve for formic acid, including carbon dioxide and hydrogen, reaches a much higher level here than it did previously.

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* See 'Roy. Soc. Proc.,' B, vol. 87, p. 87 (1917).

In the upper part of fig. 1 the hydrogen curve is seen to oscillate in a manner which is complementary to the curve for alcohol below. This constitutes the first absolute proof that alcohol is formed from glucose by reduction *in vivo.*

The formation of alcohol by reduction of acetaldehyde by means of intramolecular hydrogen was first postulated by Kostycheff for alcoholic fermentation by yeast, and later by the writer for bacterial fermentation. Still later this idea was incorporated by Neuberg in his theory of alcoholic fermentation, but, up to the present, the proof of such reduction has never been given either for alcoholic fermentation by bacteria or by yeast, although Neuberg's recent researches on phytochemical reduction have lent considerable support to the view.

The proof that alcohol is in fact formed by reduction is given in fig. 1. Moreover, it will be observed that the highest point in the alcohol curve is in the column for Experiment 3. In this case almost twice as much alcohol has been produced as in the normal experiment without added formate, so that it is clear that when formic acid is added the hydrogen which is liberated by its decomposition may also act as a reducing agent and thus augment the yield of alcohol.

As was noted in the introductory remarks to this communication, only in one out of the four experiments with formate added to the glucose was there any increase in the yield of alcohol. In the other cases there is a very marked decrease. Future experiments must decide what the conditions are which enable formic acid to act as a reducing agent in the fermentation of glucose.

Next to the proof of the participation of nascent hydrogen formed by the fermentation in the production of alcohol, the most remarkable result is the demonstration of the relation of added formic acid to the production of formic acid from glucose. The effect of the formic acid added in the form of calcium formate has been to enormously increase the production of hydrogen and carbon dioxide from the glucose.

We are accustomed to think that when one of the products of a fermentation is added at the beginning of such a fermentation the rate of production of this product should be diminished, and at the end of the experiment we expect to find a lessened accumulation of the substance in question. We should accordingly anticipate that formic acid added at the beginning of the fermentation would lessen the production of formic acid from the glucose. Now, as a matter of fact, the analysis of the solution at the end of all the experiments, except No. 1, showed that there was no excess of formic acid over that added at the beginning; but, for obvious reasons, we are not in a position to say how much of the formic acid which remained at the end of the experiment, if any, had been produced from the glucose and how much more merely represented unaltered formic acid added. The striking fact, however, is the liberation of such an increased amount of carbon dioxide and hydrogen from the glucose, for we have hitherto held the view that these gaseous products come from pre-existing formic acid. Now, in these experiments, in which glucose was fermented in the presence of calcium formate, not only the equivalent of all the formic acid which normally is formed by the decomposition of the glucose appears as carbon dioxide and hydrogen, but the total yield of these gaseous products is greatly enhanced, reaching, as will be seen in the figure in the case of Experiment 2, to nearly 50 per cent. of the glucose, a value more than double that obtained in the case of the fermentation of glucose in the absence of added formate.

How then are we to explain the fact that formic acid which itself is decomposed by *B. coli* into carbon dioxide and hydrogen, increases the production of these same gases from glucose when the glucose and formic acid are fermented together.

An attempt will be made to answer this question in Part V of this series. It seems to the writer that the answering of the question will necessitate the giving up of the prevalent idea as to the manner in which glucose undergoes decomposition in fermentation, and that it will be best to reserve the discussion until certain other facts are pointed out.

It is possible to see several important relationships from fig. 1, but it will considerably simplify the consideration of such relationships if all the curves can be considered apart from the influence of the lactic acid; for since the course of the lactic acid production runs independently of that of the other products we shall be justified in setting aside that portion of the sugar which has been converted into lactic acid, and calculating the remaining products as percentages upon the remaining portion of the glucose. This calculation has been made and the results are tabulated in Table IV. These results are further plotted in the form of curve in fig. 2 in a manner similar to that adopted in the construction of fig. 1.

The results set out in fig. 2 are arranged from left to right in order of increasing values for carbon dioxide and formic acid which after setting aside

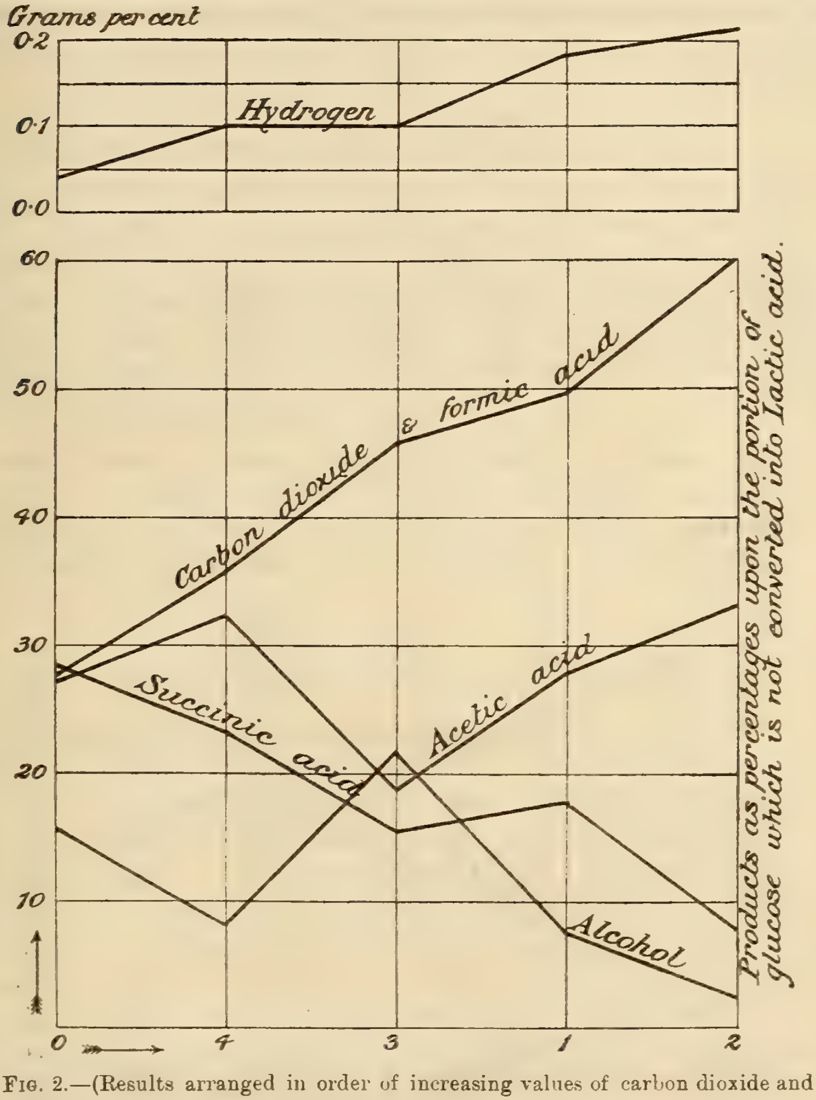

FIG. 2.—(Results arranged in order of increasing values of carbon dioxide and formic acid.)

the lactic acid is now seen to be the most variable of the remaining products from the glucose.

The immediate effect of replotting the curves after ignoring the portion of the glucose which has been converted into lactic acid is to make very clear the relationships which exist between the other products. It will be seen

Table IV.

Products of the action of *B. coli communis* on glucose fermented in the presence of formic acid. The products are calculated as percentages upon that portion of the fermented sugar which is not converted into lactic acid.

	Products derived from the glucose itself.				
	Glucose fermented alone.	Glucose fermented with formic acid.			
	0.	1.	2.	3.	4.
Hydrogen	0·46	1·75	2·10	1·01	1·05
Carbon dioxide	22·89	44·86	59·94	44·98	34·69
Formic acid	3·86	4·35	—	—	—
Acetic acid	27·03	27·48	31·86	17·70	31·37
Succinic acid	28·41	17·55	4·15	14·58	22·53
Alcohol	15·44	7·32	1·85	21·09	5·84
Total products	98·09	103·30	99·90	99·66	95·68

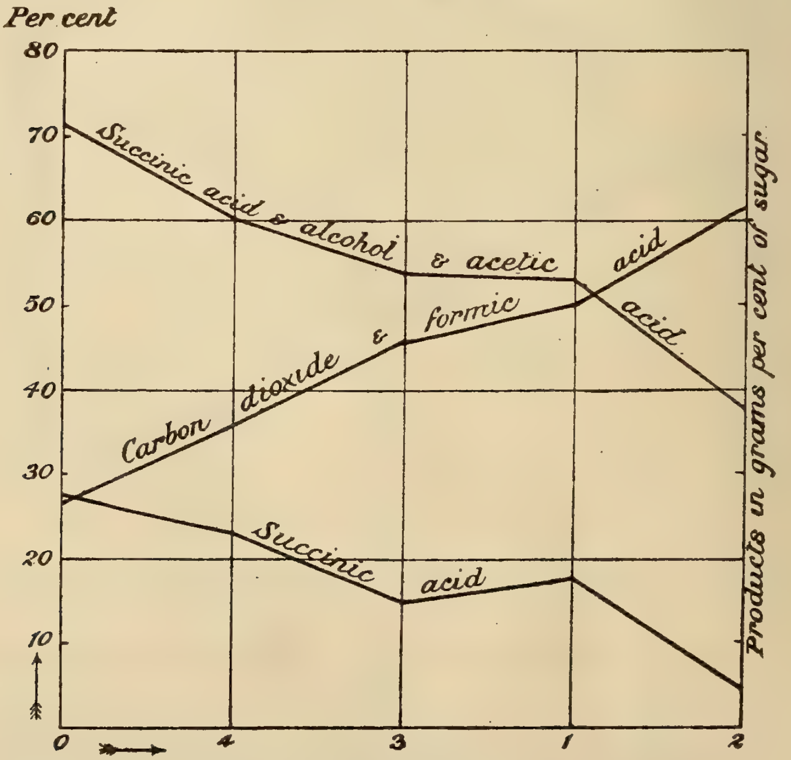

Fig. 3.—(Results arranged in order of increasing values of carbon dioxide and formic acid.)

that these products divide themselves into two groups each represented by two curves which stand in complementary relationship to one another. The acetic acid curve is the mirror image of the curve for alcohol, and the succinic acid curve is roughly the reflection of the curve representing total carbon dioxide and formic acid. In fig. 2 the succinic acid is seen to rise somewhat, opposite column of Experiment 1, whereas it should fall in correspondence to the rise in the carbonic acid curve, but reference to the figures for the total products in this experiment (Table IV) will show that in this one case they totalled to over a hundred per cent., due probably to a small amount of acid derived from the nitrogenous material of the culture medium. The important fact, however, is that the succinic acid belongs to a group of substances all consisting of two or a multiple of two carbon atoms, viz., succinic acid, acetic acid and alcohol, and that it is this group which stands in complementary relationship to the group containing formic acid and carbon dioxide. This is well seen from fig. 3.

Summary and Conclusions of Part IV.

(1) Glucose has been shown to break down under the influence of *Bacterium coli* into three main groups of products :—

(1) Lactic acid.
(2) Acetic acid, alcohol, and succinic acid.
(3) Carbon dioxide, hydrogen, and formic acid.

There is a closer relationship between the products of Groups 2 and 3 than exists between either group and Group 1. The reason for this may be that the rate at which the substances of Group 2 are formed is conditioned by the availability of the hydrogen of Group 3. If the mother substance of alcohol and acetic acid is acetaldehyde, it is easy to understand that the rate of formation of this substance would be influenced by the rate at which part of it was reduced to alcohol by the hydrogen nascent in Group 3, so that the formation of the products of the last two groups would tend to keep pace with one another, whereas the first reaction, of which the end product is lactic acid, being independent of the co-operation of hydrogen for its formation, would run a course quite independent of the other reactions, which, as a matter of fact, it is found to do.

It was shown in Parts II and III that succinic and acetic acid were closely related in origin, and this view is confirmed by the present result. Moreover, it is also clear that, in regard to the interchangeability of these two products of the fermentation, the availability of hydrogen is a critical factor. If there is no available hydrogen, succinic acid would be formed, with more hydrogen available, acetic acid, and with still more, alcohol. The proportions in which the products of Group 2 appear depends therefore on the intimacy with which the reactions of this group co-operate with the reactions of Group 1, and this further explains the fact that, in many normal fermentations, there is a tendency for Groups 2 and 3 to appear as constituting one group.

(2) It has been demonstrated for the first time in this communication that hydrogen, nascent during the fermentation, does take part in the production of alcohol. This has been shown to be true, not only for the hydrogen which arises from the decomposition of the glucose itself, but also when hydrogen is supplied in the nascent condition by the simultaneous fermentation of formic acid added to the system in the form of calcium formate. Such a proof has never been obtained before, either for bacterial or for yeast fermentation. In the case of alcoholic fermentation by yeast, although the vague expression intramolecular hydrogen was employed to indicate the idea of the participation of hydrogen in the formation of alcohol, the idea was not substantiated by any facts. More recently, some indirect evidence has been obtained by Neuberg that alcohol may be produced by the reduction by yeast of added acetaldehyde, but there is an absence of any direct proof that it is hydrogen itself which effects this reduction. Indeed, in the case of yeast fermentation, it is difficult to see how the participation of hydrogen in the process could be demonstrated, since the whole of the hydrogen of the sugar appears bound up in the final products of the reaction, whether these are alcohol, aldehyde, or glycerine. On the other hand, fermentation by means of *Bacterium coli* is admirably adapted to settle the question, for hydrogen is evolved during the fermentation, and this evolved hydrogen acts as a gauge of the amount of hydrogen which has been absorbed in the formation of alcohol.

(3) In the fermentation of glucose by *B. coli communis*, it has been shown that the effect of the presence of calcium formate is peculiar.

If carbon dioxide and hydrogen arise in the fermentation of glucose through the intermediate formation of formic acid between glucose and these products, as has hitherto been thought, then it is to be expected that the addition of formic acid to the system would tend to depress the formation of that substance, and consequently lead to a diminished yield of its gaseous products, carbon dioxide and hydrogen. In these experiments, however, the opposite has occurred. This seems to mean that either the action of calcium formate has been, by virtue of properties independent of the formic acid to which it gives rise, or else carbon dioxide and hydrogen do not normally arise by the decomposition of preformed formic acid. The writer proposes to reserve the consideration of these alternatives to Part V of this series.

(4) The method described in the present communication of carrying out the fermentation of substances in the presence of one or other of the products of the reactions added at the outset gives promise, in the writer's opinion, of lending valuable aid in the solution of the problems of fermentation.

In conclusion, I wish to express my thanks to Prof. F. Gowland Hopkins, F.R.S., for his kind help and criticism during the course of this research. I would also like to acknowledge my debt to Prof. Arthur Harden, F.R.S., for his valuable criticism.

Studies on Synapsis. II.—*Parallel Conjugation and the Prophase Complex in Periplaneta with Special Reference to the Premeiotic Telophase.*

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[Plates 10–12.]

The reality of synapsis, or the pairing of chromosomes of biparental origin preparatory to their segregation during the maturation divisions of the germ cells, provides at present the most fruitful basis for any attempt to correlate genetic phenomena with cell structure; and it rests upon data derived from sources that are strictly independent of the behaviour of the chromosomes during the remarkable series of events intercalated in the prophase of the heterotype mitosis. It is, however, only possible to gain a knowledge regarding the manner in which homologous chromosomes are brought into association with one another by a study of the meiotic phase itself. To the earlier workers the reality of synapsis was the all-absorbing problem, while the means by which it is effected formed a secondary consideration. It is possible, given the conjugation of chromosomes derived from alternate parents, to explain the independent segregation of allelomorphic pairs on the assumption that their material basis resides in different bivalents; while their localisation in identical bivalents affords an equally satisfactory interpretation of the phenomena of coupling and repulsion. Indeed were coupling and repulsion always absolute the mode of synapsis would not present an urgent cause for enquiry. The progress made of late years, however, in the investigation of partial linkages, makes it imperative for the further development of the chromosome hypothesis to postulate some mechanism for the exchange of chromatin particles between conjugating elements; so that the degree of intimacy involved in the synaptic union assumes a new significance.

While recognising with the utmost respect the brilliant speculations of Prof. Morgan and his school, it cannot be truthfully stated that there exists among cytologists at present a very high degree of unanimity as to the line along which the solution of this question must lie. In recent years the belief in telosynapsis (end-to-end union) has increased in prestige among botanical cytologists, while the alternative interpretation of parasynapsis (parallel conjugation) has found greater favour among those who have studied animal forms. In attempting to interpret satisfactorily the events of the meiotic phase in the case of animals, interest centres pre-eminently in those phenomena which intervene between the last premeiotic telophase and the formation of the "bouquet" on the one hand, and the process by which the reduced and longitudinally split (diplotene) segments become transformed into heterotype chromosomes on the other. The former furnish direct evidence of the relation of the meiotic filaments to the somatic chromosomes, while the latter in the light of the subsequent mitotic processes afford indirect data regarding the mode of synapsis, the reality of synapsis being assumed. Regarding the first, to an impartial student the work of the Schreiners (1) 1906, Jannsens (2) 1909, Agar (3) 1911, and Wilson (4) 1912, based in each case on particularly suitable material as regards both size and seriation, cannot fail to carry the conviction that here at least there is a fusion of whole chromosomes parasyndetically; and such an interpretation is fully consonant with the usual derivation of the heterotype chromosomes by separation along the line of cleavage in the diplotene filaments. There are, however, particular cases, notably among Flatworms and Orthoptera, where certain authors (Goldschmidt (5), Sutton (31), Davis (32), and Buchner (6), *e.g.*) have described the genesis of the tetrads in a manner which can only be reconciled with the theory of synapsis on the assumption that the conjugating elements are united by their extremities alone. In the case of plants the position is somewhat different; here, according to the most recent and very lucid account of Miss Digby for Osmunda, provision is made for the conjugation of univalents in the second contraction, while the fusion witnessed in the first contraction figures involves the reassociation of half chromosomes split in preparation for an arrested division in the preceding telophase. The chief issue between the rival schools in this case concerns not primarily that suggested by the etymological distinction, but the significance of the earlier stages in the meiotic phase. The telosynaptists interpret the first contraction in terms of the premeiotic prophase, whereas the parasynaptic school, like Grégoire (7), attempt to compare it with the bouquet zygotene processes in animals.

In direct opposition to Grégoire, Farmer and Moore (1905) have attempted to interpret the events of the meiotic phase in animals as well as plants in terms of the premeiotic prophases. The principal form investigated from this standpoint was Periplaneta, Osmunda being one of the forms on which the comparison was based. These two authors (8) omit any account of a leptotene or zygotene stage: their observations commence with the chromatin already present as the reduced number of loops; so that the earlier events of the synaptic processes here described are very different from those set forth in Osmunda by Miss Digby (9). According to their statement of the case the heterotype chromosomes segment transversely with respect to the line of cleavage in the diplotene stage; and since the homotype division is longitudinal, they are forced to conceive the bouquet as a spireme in which each segment corresponds to a pair of chromosomes united end-to-end. Thus we read: "the spireme threadwork tends at first to separate into half as many lengths" (as the premeiotic chromosome number). It cannot be questioned that Prof. Farmer performed a very great service to cytology in this pioneer work by demonstrating a definite relationship between the heterotype and normal somatic chromosomes. But in emphasising the similarity between the heterotype and premeiotic prophases, and more particularly the resemblances of the former in plants and animals, it appears to the present writer that the issue has been obscured rather than clarified in certain particulars, so that a re-examination of the meiotic phenomena in Periplaneta in the light of Miss Digby's discoveries would assist towards a better appreciation of the relation of these fundamental processes in animals and plants. Special attention has been directed to the study of the premeiotic telophase and its bearing on the earlier events of synapsis, a more searching study of the genesis of the spindle bivalents in spermatogenesis, and the first account of the synaptic events in oogenesis in the same form are here included. Together with these observations are recorded conclusions on the relation of the plasmosome to yolk-deposition in insects. It is a pleasant duty to acknowledge the generous encouragement and helpful criticism offered by Prof. Farmer during the course of the investigation.

Technique and Material.

Gonads from about three hundred individuals, at all stages of development from the early instars onward, were used in the present research. To ensure a plentiful supply of cells in synapsis, both testes and ovaries of larvæ were used principally, after dissection in Ringer's fluid.

Repeated trials made it clear that acid fixatives like Flemming's reagent, though admirable for work involving counts, are of little utility where the object of the investigation necessitates special attention to cleavages in chromatin filaments. All the advantages of Flemming's normal formula can be obtained by eliminating the acetic component, so that swelling of chromatin is avoided. Gatenby has already recommended this modification for the study of mitochondria. Very successful results were obtained by a modification of one of Rabl's formulæ: equal parts of formol and 1 per cent. platinic chloride were mixed, and diluted with two volumes of distilled water. The mixture penetrates admirably; and, while the chromatin elements shrink too much for convenient metaphase counts, for the examination of filaments, where cleavage is doubtful, it gives an admirable fixation. In all cases where iron hæmatoxylin was afterwards used, the sections were previously stained with a transparent dye, and ultimately differentiated in dilute alum very slowly under constant microscopic observation until the pigment was all but removed from the object studied. Such precaution is essential; in Periplaneta, the elongated annular heterotype chromosomes appear in Haidenhein preparations (owing to the elimination of the interspace by adsorption of stain) as homogeneous rods, long after all the dye has been extracted from the chromatin of resting cells. The importance of care in staining is sufficiently evident in the illustrations of authors (*e.g.*, Buchner) who have used the iron method.

1. *Oogenesis in P. americana.*

As in other Orthoptera, the ovarioles of the Blattidæ are composed of only two kinds of cellular elements, follicular cells and oocytes; there are no nurse cells. In the imago, four regions may be distinguished in the egg tube: (*a*) a portion composed of oocytes in linear series, surrounded individually with follicular epithelium, and occupying altogether about four-fifths of the entire length of the tube; (*b*) a region of oocytes not as yet arranged in linear series nor separately invested with follicles, but already displaying the nucleus in the *diffuse* condition characteristic of the growth phase; (*c*) a zone of young oocytes in synapsis and of oogonia with large spherical nuclei; (*d*) the terminal filament, consisting of smaller cells, with inconspicuous and granular ovoid nuclei. Neither in imagines nor in early instars could any evidence of transition between cells of the terminal filament and the large oogonia of the synaptic zone be established, though the relation of the terminal filament and chamber in the Hymenoptera and some other insects is manifest; from a comparison of the number of eggs in the remainder of the ovariole and the number of mitoses observed in the synaptic zone, and also, after consideration of the number of eggs normally laid during the period of sexual activity, it would appear that the terminal filament in the Blattidæ does not contribute to the formation of new oogonia. The origin of follicle cells in *P. americana* is particularly easy to recognise, owing to the fact that the follicular chromosomes assume in the prophase the atelomitic (U-shaped) form which they display on the equatorial plate, thus contrasting sharply with the short stout elements of the oogonial complex; they arise from the oogonia themselves. This has also been shown to be the case in the Hymenoptera by Paulcke (10) and the author (11), both nurse and follicle cells being of germinal origin; whereas, in some Diptera, *e.g.*, the Cecidomyid Miastor, the oocytes alone are derived from the primordial germ cells.

In view of recent work on the meiotic phase in plants, a critical survey of the synaptic events necessitates a thorough appreciation of the character of the premeiotic mitoses. A very full description of the process as it occurs in the archesporial divisions of Osmunda is thus epitomised by Miss Digby: "In late anaphase the substance of each chromosome is seen to be divided already into longitudinal halves. As the newly formed nucleus proceeds to rest the chromosome halves become beaded. The threads gradually separate widely, and the beads become resolved into finer and finer granules until a fine reticulate edge is reached—the so-called resting stage. During prophase the series of events is reversed and the chromosome halves or threads reassociate . . . . The association of the two halves becomes increasingly intimate until it results in the organisation of the completed univalent chromosome. . . ." It is a singular fact that in Periplaneta the overwhelming majority of oogonia, either in adult or pre-adult ovarioles, are always seen to be in the prophase, when the chromatin elements are represented by prochromosomes in various stages of development. In the metaphase of the oogonial divisions the number of chromosomes is clearly thirty-four; they are stouter than those in the male germ cells, and uncurved. It is evident that the representative of the accessory chromosome described in the male of the species by Morse (12) is accompanied in the division of the female germ cells by a homologous partner. For the sake of brevity it may be remarked in this connexion that the accessories in *P. americana* do not behave in any stage of the nuclear history of the oogonia in a manner distinguishable from the autosomes.

By the examination of sufficiently thin sections it is possible to trace the behaviour of chromosomes in their passage to the polar end of the spindle with accuracy and assurance (*cf.* figs. 4 and 20); and it is clear that in the later oogonial divisions, at least, the telophasic chromosomes undergo no cleavage. This conclusion is fully consonant with succeeding events. It is possible that the telophase chromosomes, in some cases, pass over directly to prophase prochromosomes without the intervention of a reticulum: all that can be legitimately inferred from the extraordinary rarity of typical resting nuclei is that the reticulate condition must occupy a very brief portion of the nuclear cycle. In the prophase the reticulum condenses into cloudy areas that indicate the future chromatic elements; and in elucidating the genesis of the latter, comparison with the nuclei of the incipient follicle cells is valuable in view of the closer resemblance of the chromosomes of the latter to the curved units of the male complex. There is in neither case any sign of an "increasingly intimate" reassociation of telophasic half-chromosomes for the "organisation of a completed univalent" as described in the archesporial nuclei of Osmunda. On the contrary, the cloudy area which is, in the first place, without any sign of division, gives place to a more definitive chromatin mass, which does not display cleavage until the characteristic form of the metaphase unit is already recognisable. Stages in the transformation are represented in Plate 10, figs. 1–3 and 5–7. A comparison with corresponding events in the male germ cells will be made later when further details have been supplied under that heading.

The Meiotic Phase in the Oocyte.

According to the account already quoted, the earlier events of the heterotype prophase of Osmunda, involved in the first contraction, are primarily concerned with the reassociation of the telophasic threads (half chromosomes) as in a normal prophase; in fact, they are equivalent to an arrested but in other respects normal prophase. It is clear, then, that Grégoire's attempt to compare the first contraction of plants with the parasyndetic bouquet is rendered invalid: indeed, the question of the parasyndetic interpretation of the zygotene stage in animals itself assumes a more problematical aspect. To ascertain whether in the light of what occurs in Osmunda, the bouquet stage of animals like the first contraction figure in plants is amenable to interpretation in terms of the normal somatic prophase, "it is evident," as Wilson pertinently observes, "how essential it is to determine the number of presynaptic threads, for if they have such an origin as has just been indicated, their number should be tetraploid, whereas if they represent whole chromosomes, their number should be diploid."

As already stated, there is no evidence of cleavage in the telophase of the later oogonial divisions studied; and a preliminary examination of the early nuclear history of the oocyte made it abundantly evident that stages which are obscure and difficult to interpret in the male sex cells display a remarkable clarity in the ovarioles, where the fixation is facilitated by the small diameter of the terminal chamber. In the earliest stages (fig. 8) the nucleus of the oocyte is readily distinguishable from the confused reticulum of the oogonial resting nuclei by the organisation of the chromatin as a dense tangle of finely beaded, single, convoluted and none the less definitive threads which at first exhibit no sign of polarisation, and lie in contact with the nuclear membrane; this condition corresponds with what is now generally described as the early leptotene stage. The paired accessory chromosomes which are indistinguishable from the autosomes on the equatorial plate of the oogonial mitoses behave, as will be seen later, like their fellows in the ensuing stages; in this respect they resemble those of the Orthopteran Leptophytes recently described by Mohr (13).* Very soon the irregularly disposed filaments of the early leptotene stage assume the characteristic polar arrangement which marks the formation of the bouquet (fig. 9). In the early bouquet the filaments are seen in the form of loops polarised at both ends; and not as yet withdrawn from the nuclear membrane. In the preparations examined a number of such nuclei were cut in the plane at right angles to the axis about which the leptotene loops are orientated; and it was thus possible to count the number of the latter, each of which would in median section be cut twice. In several cases the number of points was so clearly between sixty-six and sixty-nine, as to leave no doubt that the full diploid number is here represented (fig. 10). The attenuated, single and finely beaded filaments of the early leptotene bouquet now contract away from the nuclear membrane become shorter and more closely approximated. As the process of contraction continues, the filaments are brought into closer union in pairs, and it appears that the paired association of the loops begins at the polar ends, extending to their distal extremities, until the whole bouquet is formed of intimately associated loops (fig. 11). By the time maximal contraction has been effected the interspace between the conjugating filaments is hardly recognisable; but in the best preparations it can be seen that even in this stage, the *pachytene* threads retain their double character with sufficient clarity to indicate the nature of the cleavage which follows (fig. 12). When it is borne in mind that most of the available data relating to the synaptic phase in animals has been supplied by authors who have been more concerned with the chromosome complex in the germ cycle, it is perhaps pertinent to ask whether the "synizesis" figures described again and again (as in a previous study on Hymenoptera) would not present a less homogeneous appearance with more specialised technique. It is certain at least that in the case under consideration, it is easy enough at the point of maximal contraction to obtain typical synaptic knots with over fixation and logwood staining. Maximal contraction does not seem to extend over a long period in *P. americana.* As the loops widen out, the interspace between the constituent halves of the thickened more chromatic loops of the pachytene stage again becomes easily visible (fig. 13). It is easy to see both in this and the preceding stage from sections at right angles to the orientation of the loops (fig. 15) that the reduced number of loops is now present. The longitudinally divided diplotene loops become considerably elongated, and enter into contact with the nuclear membrane; but again become shortened somewhat before the complete dissolution of the bouquet figure (figs. 14 and 16).

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* *Cf.* Winiwarter and Sainmont (34).

Up to this point the facts briefly stated are as follow: there is no cleavage of chromosomes in the telophase of the oogonial mitosis; the reduced number of thickened loops in the stage of maximal contraction is brought about by the parallel conjugation of leptotene threads equivalent in number to the telophasic chromosomes of the preceding mitoses. Provided that such an interpretation is not incompatible with the observed transformation of diplotene loops into heterotype chromosomes, it is difficult to escape the conclusion that the process described is not analogous in any way to a premeiotic prophase, but an event *sui generis* in which the conjugation of homologous chromosomes is effected. It is therefore necessary now to enquire whether such a conclusion is consonant with the subsequent events. But before doing so, it may be stated that there was no evidence of the twisting of the filaments in the zygotene stage; indeed, while such a procedure does not seem improbable in the case of leptotene filaments that are polarised at one extremity only, it is difficult to imagine in the case of paired loops, apparently attached at both ends, any mechanism by which such twisting could be achieved. The union of the constituent halves of the pachytene loops is sufficiently intimate to allow the possibility of "crossing over"; but the facts do not seem to justify the belief that this is brought about by such means as are postulated by the chiasmatype theory.

The free diplotene loops represented in fig. 16 rapidly become abbreviated and straightened, a process in which two, however, lag behind their fellows precisely as do two of the loops in the heterotype prophase of the male sex cells (figs. 17, 18*a*). One of the bivalent filaments of this stage is distinguishable by its singular form, and smaller than the rest; it is possible that it represents the accessory pair (fig. 18*b*). The constituent halves of the bivalents now become widely separated in the region midway from the extremities of the filament (fig. 19). And as the confused stage supervenes, the axis of each filament becomes shorter, the interspace widens, until each half is separated along its entire length from its fellow, becoming contemporaneously more sharply curved and more granular in appearance. Thus the bivalent elements pass into the reticulate condition characteristic of the growth phase of the egg as transversely divided rings, such as are described on the heterotype spindle of the male by Farmer and Moore. But it will be observed that their mode of formation is very different: the ring is formed by the opening out of the diplotene cleavage, and not by the union of loops by their free ends as described in Farmer and Moore's account of the spermatogenesis of the species. The validity of the alternative interpretation will be fully discussed in dealing with the reduction divisions in the male. Since, in general, the bivalents emerge from the diffuse stage in the same form which they exhibit before its onset; and since the heavily yolked ova of Periplaneta did not yield any critical data regarding the details of polar mitosis the nuclear history of the oocyte may be here concluded, by observing that as the bivalents become more granular in form (fig. 21) they merge into reticular units, eventually losing their visible individuality altogether. As in other insects, the cytoplasm of the oocyte from this point onwards undergoes enlargement. This stage also synchronises with other events in the egg: firstly, the mitochondria which are well preserved in unacidified chromosmic preparations cease to congregate around the centrosome as in the young oocytes and oogonia (fig. 8), migrate from the neighbourhood of the nucleus, and become dispersed uniformly in the surrounding cytoplasm; secondly, infection by the symbiotic bacteria (*Bacillus cuenoti*) described by Mercier (14), and indicated in fig. 28, usually occurs at this point, a well-defined zone of symbionts being seen in the periphery of the egg by the time it assumes a definite outline (fig. 22); finally, the plasmosome is seen to undergo changes to be described hereinafter.

In concluding this account of the nuclear history of the ovarian oocyte, attention may now be directed to the criticism of those opponents of parasynapsis, such as Meves, Fick, and Duesberg, who, while emphasising like the telosynaptic school the importance of an understanding of the premeiotic prophase, appear to recognise that the interpretation of the early events of the heterotype nucleus in terms of a normal prophase can only be accepted for animals by rejecting the doctrine of synapsis itself. Duesberg after affirming, "un prémier élément absolument indispensable à sa solution (the problem of numerical reduction) c'est la connaissance approfondie des prophases de la mitose somatique," passes on to explain the parallel fusion, so often described, in the zygotene stage, in the following terms: "les grosses travées ne se forment pas par fusionnement latéral de deux fins filaments, mais se produisent de la façon suivante, certaines travées reçoivent la chromatine qui charge les travées sécondaires . . . . c'est le parallélisme des filaments chromatiques qui produit les images qui ont été interpretées comme un fusionnement latéral de chromosomes . . . ." (15). As a statement of fact, this has been sufficiently dealt with by Wilson. Let it be admitted nevertheless that "une connaissance approfondie des prophases de la mitose somatique" is an essential condition for the correct interpretation of the meiotic phase; let it be conceded, moreover, that many advocates of parasynapsis have paid too little attention to such phenomena: on what facts does Duesberg base his comparison of the somatic and maturation prophases? Obviously, there are three valid criteria: similarity in shape, number, and dispositions of the chromatin units. As regards the first, nothing could display a more marked difference from the gross bacilliform chromosomes of the prophase of a normal mitosis and their compact granular precursors in the condensation of the reticulum than the attenuated and finely beaded loops seen in the heterotype nucleus from its very inception; there is evidently no basis of comparison to be sought in this direction. Turning next to the numerical relations, Duesberg disdains to consider the witness of numerous observers to the existence of the full diploid number of filaments before synapsis; it is, therefore, not surprising that he fails to appreciate the dissimilarity of the zygotene stage to any phenomenon characteristic of the nuclear history of the premeiotic germ cells. When finally the orientation of the bouquet, so common in representatives of the most diverse groups of animals (flat worms, insects, molluscs, fishes, amphibia, and mammals), is taken into consideration, Duesberg can only protest that in a few cases a typical bouquet is absent. This is admittedly true; but to deny a universal polarisation of the zygotene filaments does not invalidate the statement that the disposition of the chromatin elements in the early stages of the meiotic phase is altogether unlike their arrangement in a typical prophase nucleus. In Periplaneta the premeiotic and maturation prophases resemble one another neither in the form, numerical behaviour, nor disposition of the chromatin units: the maturation prophase is quintessentially concerned with bringing about the conjugation in pairs of filaments which are individually equivalent to the chromosomes of the antepenultimate telophase of the germ cell divisions in the individual.

It is difficult to see why it should be regarded as more scientific to interpret the phenomena of mitosis in terms of military strategics than in a manner by no means in conflict with the data, and fruitful as a working hypothesis towards a knowledge of the morphological basis of inheritance; without, however, entering here upon a discussion of the ingenious "manœuvre hypothesis" of Fick, it may be allowed that his followers have at least a plausible and even legitimate case when they criticise the doctrine of synapsis on the ground that there is as yet no *direct* general evidence for the persistent individuality of chromosomes. When, on the other hand, they endeavour to show that the early events of the meiotic phase conflict with a belief in the reality of synapsis, their interpretation can only be described as a travesty of the facts recorded by a very large and increasing body of investigators.

The Formation of Yolk.

A single nucleolus is present in the oogonia and in the oocyte from its inception. In the earliest stages it can be readily identified as a plasmosome, with Auerbach or methylene blue-eosin staining methods (fig. 10). At first spherical, it becomes enlarged and temporarily pear-shaped about the time when the nucleus passes into the diffuse condition, but it speedily reassumes a characteristically spherical shape. From the beginning of the growth phase it is seen to emit minute deeply staining particles, which appear to pass bodily through the nuclear membrane and migrate to the outer boundary of the cytoplasm, where they are either ejected or transformed into some material no longer distinguishable from the ground cytoplasm itself (figs. 21 and 22). At a certain point, which has, as will be seen later, a definite relation to the deposition of yolk, a noteworthy series of changes commence. In Haidenhein preparations of material fixed without acetic acid, the plasmosome has hitherto displayed a markedly chromatic appearance; it now loses its opacity, and vacuoles are recognisable in its substance (figs. 24–27). At a later stage these vacuoles assume a granular and more chromatic form, simulating, in fact, miniature nuclei within the ground substance of the nucleolus. The structure of the nucleolus at this stage is represented in optical section in figs. 23, 28, and 29. From now onwards, appearances suggest that the ejection of the more minute and uniformly chromatic particles alluded to above ceases, and the vacuolar bodies within the plasmosome are cast out into the karyolymph, boring their way, as it were, through the nuclear membrane, eventually to periphery of the egg (fig. 31). As these intranucleolar bodies, which, for reasons that will be explained, may be termed *deutosomes*, are discharged in this manner, new vacuoles appear within the plasmosome, and a similar metamorphosis ensues, until the deposition of yolk is nearing completion.

In examining preparations of the eggs of Periplaneta, the observer cannot fail to be impressed with two facts, namely, that the inception of yolk formation, on the one hand, bears no relation to the size and shape of the egg, while, on the other, the first appearance of the deutoplasmic spheres clearly synchronises with the vacuolation of the plasmosome and the changes consequent thereon. The first indication of the onset of yolk deposition is the formation of vacuolar areas in the periphery of egg; within these are often seen the minute chromatic granules first emitted by the plasmosome, as already described. An exactly similar process precedes the formation of the remarkable secondary nuclei of certain Hymenoptera, described in a previous communication (11). True secondary nuclei, however, are not formed in the Orthopteran species examined (fig. 22). Nor do these events appear to be directly related to the formation of the deutoplasmic spheres. The first yolk is deposited at the outer boundary of the egg in the form of spherical globules that are not homogeneous, and are, in fact, both as regards size and appearance, precisely like the intranucleolar *deutosomes.* The latter break up into several homogeneous globules, which are the yolk spheres *sensu stricto* (fig. 31). Thus it seems probable that the nuclear appearance presented by the intranucleolar bodies is the result of adsorption to the surface layers of an internally heterogeneous mass in process of dissolution.

That the plasmosome stands in an intimate functional relation to the formation of yolk will not be regarded as improbable by those who have studied oogenesis in insects, and many investigators of this problem have recognised the synchronism of yolk formation and the ejection of nuclear granules. In tracing the yolk spheres of Periplaneta to an intraplasmosomal origin, the present author has not arrived at this solution without considerable diffidence, and, were it not for the possession of a very complete series, would hesitate to advocate such a conclusion. It may be said, however, that very similar events were observed in certain Hymenoptera, although no account was given, in view of the desirability of examining more favourable material with more suitable technique. The seriation of stages in Periplaneta is facilitated in this matter by the independence of deutoplasmic differentiation and cytoplasmic bulk, while the relation of the former to the vacuolation of the plasmosome is too striking to escape notice, even when the attention is primarily focussed on other questions.

From the heterochromosomes in the synaptic period and the facts elucidated above, it might well be surmised that the accessory body described by Buchner (96) in Gryllus as the equivalent of the male heterochromosomes is in reality a plasmosome. To test this possibility, the oogenesis of *Gryllus domesticus* was investigated, and the results justified anticipation. While insufficient material was studied to yield a complete account of the synaptic phase, it may be added that follicular mitoses clearly indicate that the heterochromosomes of the female are paired. No more need be said on this matter, since Gutherz (16) has denied the authenticity of Buchner's observations, and Mohr has based a very destructive criticism of Buchner's theory of the trophic function of accessory chromosomes on his own researches into the oogenesis of Leptophyes (13). An inspection of Buchner's figures of badly fixed preparations, evidently overstained in iron hæmatoxylin, will suffice to vindicate Vejdovsky's (17) comment that his "Angaben und bildlichen Darstellungen der ganze Reifungsperiode der Grylluseier sind ganz wertlos."

A point of more general significance, however, arises in this connection. Various authors appear to assume that the nuclear (so-called chromatin) particles, formed during the growth period of the egg and ejected into the surrounding cytoplasm, are derived from the chromatin reticulum; and the question of the bearing of these phenomena, on Boveri's theory of persistent individuality, has recently been raised by Gatenby (18), according to whom these particles in Apanteles are identical in all their reactions with chromatin itself. It might be pointed out, in reply, that chromosomal structures themselves are not identical in all their staining reactions, and the existence of a technical diagnosis of "chromatin" by staining is doubtful. It is well known that physical differential staining is a process in which a large number of factors participate, such as the elasticity, permeability, size, and fixation of the material, the diffusibility and concentration of the dye, the period over which and the temperature at which the experiment is conducted. Thus substances of identical chemical constitution can be differentially stained, while conversely dissimilar compounds may be dyed alike. Clearly, then, *the only crucial test available is continuity or discontinuity of structure.* The suggestion is here advanced that further study would reveal the origin of the so-called chromatin granules emitted from the nucleus at the diffuse stage in the plasmosome of forms other than Periplaneta, and a reconsideration of previous preparations confirms this belief, as far as the Cynipids are concerned.

As one writer on the subject has commented felicitously, chromatin is a morphological rather than a specific chemical substance: morphologically there is no adequate evidence of continuity of structure between the chromosomes and the plasmosome, directly or indirectly, as a study of the telophase demonstrates. It is for those who like Schaxel (19) speak of the part played by chromatin in the genesis of the oocyte to furnish such evidence. Nevertheless the demonstration of the chromatin origin of the nuclear granules which appear to be extruded—and the onus of proof lies with those who make the affirmation—would not be a fatal blow to Boveri's theory; for those who advocate a structural continuity of substance between individual chromosomes do not necessarily conceive chromatin as a static constituent of the cell: if the ultimate mechanism of inheritance is conceived to reside in the autocatalytic properties of the substance of the chromosomes, the elimination of chromatin raises no special objection. A study of the conditions under which the cell structures hitherto described as nucleoli are formed and their relation to the chromatic organisation of the nucleus would perform a genuine service to cytological theory.

Chubb (20) has described the relation of the nucleolus to the deposition of yolk in the Echinoderm Antedon; and in many points his observations display similarity to those set forth above: but it must not be inferred that the data supplied by the examination of fixed material necessarily furnish a faithful facsimile of the actual process as it occurs in the living organism. In the absence of further evidence derived from ultramicroscopical examination of fresh tissue, it can only be legitimately inferred that a transportation of material from the nucleolus is instrumental in the formation of the deutoplasmic spheres. It is necessary to state this because of the difficulty of imagining the movement of comparatively large bodies in viscous media according to such a definite mode of procedure. Whether then the gemmules correspond to fluid globules or solid particles of corresponding magnitude, or whether they are to be regarded as precipitation products of a diphasic colloidal system, cannot be definitely stated. There are, however, two considerations which uphold the former alternative, namely: (*a*) the constancy of magnitude exhibited by these bodies after precipitation by a variety of reagents (*vide infra*); and (*b*) correspondence in size, when seen in media of apparently different density (nucleolus, karyolymph, and cytoplasm).

The nucleolar particles observed in Periplaneta are *not* identical in their staining reactions with the chromatin network; but for the reasons stated emphasis will not be laid on this point. As regards the logwood dyes, Haidenhein after fixation with picroformol-acetic (Bouin), bichromate acetic (Tellyesnicsky), chromosmic acetic (Flemming), caused the plasmosome in the very young oocyte to stain deeply like the reticulum: in later stages, as figured, the chromaticity of the former appears to diminish, giving a translucent greyish effect immediately before the formation of the deutosomes. After acid-free fixation—chromosmic (Gatenby)—the plasmosome was seen to be deeply chromatic at all stages, when stained with iron hæmatoxylin, but translucent and less chromatic than the reticulum at all stages after treatment with hæmalum. In all these cases the early nucleolar particles were deeply stained by the dye. With methylene blue-eosin staining the plasmosome and nucleolar particles and gemmules were basophil, and indistinguishable from the reticulum after acid fixation, but acidophil and sharply differentiated from the network after fixation by Gatenby's method. Auerbach's fuchsin methyl green after sublimate acetic treatment gave a definite differentiation ; but after acid-free fixation acidified Ehrlich Biondi's methyl green-fuchsin-orange G failed to give the characteristic chromatin reaction.

2. *Spermatogenesis in P. americana.*

The spermatogenesis of the Blattidæ has been dealt with from various aspects by la Valette St. George, Von Rath, Erlanger, Stevens and Wassilieff; but as none of these writers have been primarily concerned with matter under consideration—the problem of synapsis—it is hardly necessary to include further reference to their work. Farmer and Moore, '05, and Morse, '09, on the other hand devoted their attention principally to a description of the reductive processes; and as will be seen later, their conclusions are signally lacking in conformity on most points of crucial interest. Neither of these investigators paid any attention to the oogenesis of the same species, though as already shown the early events of the meiotic phase can be there witnessed with remarkable clarity and precision. Owing partly to the greater width and adiposity of the testes as compared with the minute ovarioles of the larvæ, it is difficult in the former case to ensure a sufficiently rapid penetration to preserve the contraction stages in good condition, and even with smear preparations which are subject to instantaneous fixation the constitution of the *bouquet* presents a very much more difficult problem in the male sex cells than in those of the alternate sex. Nevertheless there remain certain points for the elucidation of which the spermatogenesis offers more favourable material for study than oogenesis. By securing a supply of testes from individuals that have not attained to sexual maturity it is possible to obtain plentiful sections from cysts exhibiting every stage between the anaphase and the formation of the spermatogonial reticulum. Later instars likewise provide a greater proportions of cysts with heterotypic prophases and metaphases than will be found to occur in the testes of the adult. Special attention will therefore be paid in this section to: (*a*) the character of the premeiotic telophase in its bearing on the heterotypic prophase; and (*b*) the means by which the diplotene loops are transformed into the mature spindle bivalents.

The Premeiotic Telophase.

That the oogonial chromosomes do not exhibit cleavage either in the telophase or earlier in the anaphase is very certain in Periplaneta; but since mitoses are rare in the ovarioles full details as to the genesis of the nuclear reticulum are difficult to obtain. In Farmer and Moore's account of the spermatogenesis of this species the events of the premeiotic telophase are not described; while Morse figures at this stage a homogeneous mass of iron hæmotoxylin which hardly sheds any further light on the question. In general it may be said that zoological writers with very few exceptions have paid little attention to it, though in the case of plants it appears that a proper understanding of the premeiotic telophase provides the key to the earlier processes of synapsis. It is obvious that to attempt a recomparison of the maturation prophases in animals and plants, it is above all things essential to gain such an understanding. The clumping together of the chromosomes of the spermatogonial telophase shown in the illustrations to so many papers, is in the opinion of the present writer purely an artifact due to bad fixation, inappropriate staining, and in general to a failure to recognise that almost every stage of cell history requires separate technical attention. All acid fixatives, and in particular the sublimate mixtures, produce a swelling, which is admittedly advantageous in estimating the chromosome numbers of metaphase plates, where each chromosome is widely separated from its neighbours; but as chromosomes pass to the poles of the spindle, they become approximated more closely, so that it is desirable to use acid-free mixtures such as platinic formol, which produce a shrinkage rather than a swelling of the individual elements. By such methods it is possible not only to recognise the telophase chromosomes individually but to ensure that any evidence of cleavage which would be obscured by swelling does not escape observation. One further point should be added, it is useless to study the telophase in Haidenhein preparations; a transparent stain such as gentian violet (corrected with a light filter) is to be preferred.

The arc-shaped chromosomes of the spermatogonial metaphase display median fibre attachment until the anaphase; and no indication of cleavage is to be seen (figs. 32, 33). On reaching the poles of spindle the chromosomes become arranged with their long axis in the plane that is parallel with the axis of the spindle itself. This circumstance greatly facilitates the study of their behaviour, in sections transversely cut with reference to the spindle, the telophase chromosomes appear as points, the number of which is about thirty-two (*cf.* figs. 33, 34). The appearance of the nuclear membrane precedes the dissolution of the spindle, though no spindle remains are found in the typical resting cells. At this stage the individual chromosomes become attenuated, at first retaining their parallel arrangement; and in this condition as well as in the succeeding stage, when they become scattered in the nucleus without orientation with respect to the spindle, it is possible to make quite certain that they remain undivided (fig. 35). The scattered thread-like telophase chromosomes then become more granular, less definite in outline, assuming a zigzag delineation, in which condition they merge into the reticulum (fig. 36).

The process by which the prophase chromosomes are built up has been fully described by Farmer and Moore. It is described by them in the following terms: "mitosis is ushered in by the increasingly chromatic appearance of the cells . . . . at first the cells which are preparing for division present an even granulation of the chromatin within their nuclei, and this in its consistency strongly suggests a foam structure of the ordinary type; but after a time the chromatin confusion, as it were, sorts itself out into obvious condensations or flocculent areas, and it is apparently unquestionable that each of these clouds is individually the forerunner of one of the future chromosomes. The gradual condensation which occurs in each cloud proceeds moreover in such a manner that the chromatic granules become arranged in two distinct rows or tracts. So that by the time the individual chromosomes have attained to some sharpness of definition they appear also as if they had been longitudinally split from end to end." Apart from the negative generalisation that there is no stage at which the prochromosomes are undivided, this account is unexceptionable. That the flocculent prochromosomes of the prophase are, however, at first undivided, can be shown in smear preparations, as would be expected from what occurs in the preceding telophase.

In any case, the premeiotic divisions of Periplaneta, unlike the archesporial divisions of Osmunda, do not show an increasingly intimate association of half-chromosomes, resulting in the organisation of the complete univalent chromosome in anticipation of the metaphase; on the contrary, the cleavage of the chromosomes in Periplaneta exhibits progressive differentiation, beginning in the inception of the prophase itself. This distinction is doubtless correlated with the different behaviour of the chromosomes in the telophases of the two cases; it may therefore be said that behaviour of the chromosomes in the telophase of the premeiotic mitosis amply confirms the conclusion that each loop of the leptotene bouquet is equivalent to a whole univalent. The modification of the process which occurs in the last premeiotic division is correctly described by Morse.

Synapsis.

Farmer and Moore commence their account with maximal contraction. No conflict arises therefore between their interpretation of the mode of synapsis derived from the formation of the heterotype chromosomes and the processes involved in the elaboration of the synaptic bouquet. Morse, on the other hand, gives data less detailed but essentially in unison with those already described in the early history of the ovarian oocyte. The polar orientation is preceded by a tangled leptotene stage, in which the individual filaments are only traceable with great difficulty: as polarisation sets in, optical sections reveal the presence of about thirty, *i.e.*, the diploid number of threads; later, as described by Farmer and Moore, only sixteen thickened loops are seen. Throughout these stages the unpaired accessory is recognised as a fusiform body closely applied to the plasmosome, and hence confused with that body by Moore and Robinson (21). Morse does not describe the zygotene stage, but concludes from the arrangement and numerical relations of the threads before and during maximal contraction that parallel conjugation occurs. In good transparently stained preparations evidence of the actual assembling of the loops in pairs can nevertheless be traced, though this is admittedly difficult, owing to the compact state of the chromatin filaments. Fig. 56 in Farmer and Moore's paper suggests a zygotene stage in which the interspace between two diverging loops is too much stained to allow the line of separation to be manifest.

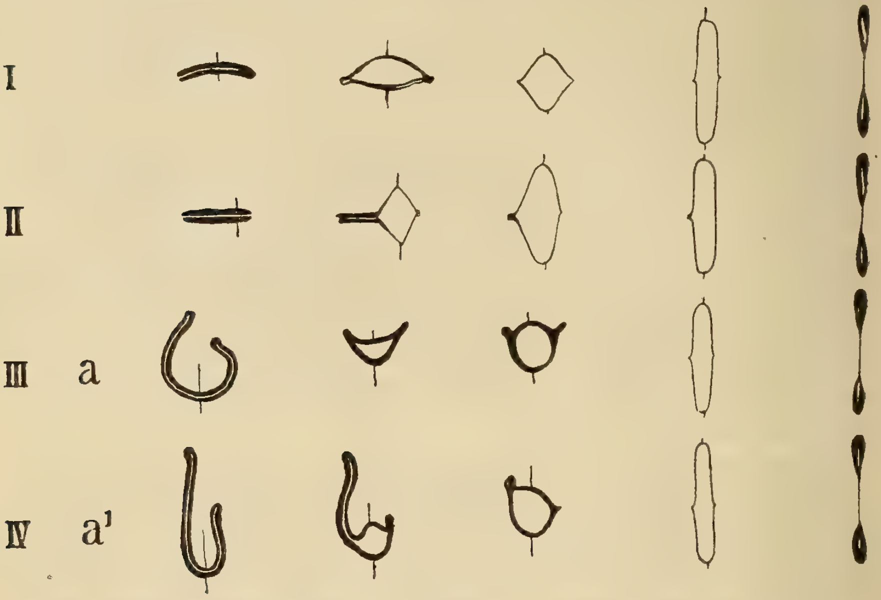
In the unravelling of the postsynaptic spireme the diplotene threads undergo a very complicated metamorphosis that cannot in the least be regarded as a typical meiotic phenomenon: they first become extended and later shortened, so that Farmer and Moore speak of a second contraction figure. This is not in the strict usage of the phrase "a contraction figure," because the threads lie manifestly on the nuclear membrane, though they happen to be concentrated towards one pole of the nucleus. At a still later stage, as Morse figures, half of the loops are seen at one pole and the remainder at the opposite one, this condition being preceded by the division and separation of the centrosome in preparation for the ensuing heterotype mitosis.

A Sequential Analysis of the Heterotype Complex.

It has been seen in the oocyte that after the dissolution of post-synaptic spireme the diplotene loops become straighter and shorter. In the formation of the heterotype chromosomes of the male, however, according to Farmer and Moore, the loops of what they term second contraction separate and immediately unite by their free ends to form ring-like chromosomes which become stretched lengthwise of the spindle and divide transversely. Two rings in their account remain open at one side and retain evidence of the diplotene cleavage; they are stated to divide in the same way as the remainder. According to Morse, on the other hand, the polarised loops straighten out and proceed to division in the following manner: "The chromosomes come to lie in the equator of the cell with their longitudinal axes in that plane. Some of the bodies precociously arrange themselves for division, while others lag behind. *For this reason one cell may present an almost complete history of the division of the chromosomes.* The attachment of the spindles fibres. . . . may be in the middle thus pulling the daughter chromosomes apart symmetrically to form a ring; or the attachment may be nearer one end of the chromosome than the other, so that the daughter chromosomes are pulled out at first into a bracket figure . . ."

These two descriptions, the one telosynaptic, the other parasynaptic, are needless to say mutually exclusive; moreover, neither is adequate. The inadequacy of the last is indicated by the sentence italicised, since if the chromosomes of the heterotype mitosis differ *inter se*, as regards the actual form of the cleavage, a complete history can obviously only be obtained by tracing out the genesis of each chromosome individually. As regards Farmer and Moore's interpretation, it is only necessary to say that they only figure two chromosomes giving any indication of the method of transformation which they describe in the text.

A sequential analysis of the chromosome complex in the heterotype metaphase is a matter of great difficulty, where the number of chromosomes is as large as in Periplaneta, and since it may be stated without exaggeration that of the sixteen bivalents, no two pass through all stages of the transformation from the diplotene threads (and loops) synchronously, space does not permit the illustration of a complete series of nuclei demonstrating every detail of the transition from the post-synaptic bouquet to the heterotype telophase. In the present investigation it was possible to obtain a series of reconstructions of whole nuclei representing some fifteen stages: in the interpretation of these a certain element of error may arise in consequence of the fact that the form attributed to a particular chromosome of necessity depends on whether it is superimposed upon another, the plane in which it is viewed, and like considerations. So that no single cell from section or smear preparation is adequate for a given stage, while it is not always possible to find another cell at exactly the same stage to settle a doubtful point. However, after a prolonged and somewhat tedious comparison of a very large number of nuclei, it is possible to record with assurance the main facts. At the inception of the metaphase fourteen out of the sixteen bivalents are rod-shaped or arc-like, the remaining two being much more attenuated and still retaining the looped condition in which the diplotene threads emerge from the bouquet stage. These latter will be called the *a* and *a′* chromosomes, and it will be remembered that there is a similar lagging behind of two of the heterotype elements in the oocyte described earlier in this paper. The order and method of division of the bivalents is as follows: (1) the first seven divide longitudinally by median fibre attachment, being drawn out into elongated rings which are segmented transversely; (2) of the next seven, the first three divide with subterminal attachment, while of the remaining four, it appears that three probably show median fibre attachment: Morse's belief that these chromosomes which are attached to the spindle subterminally are drawn out into bracket-like figures was not confirmed: the best preparations show that these too give rise to stretched rings of the usual type; (3) the *a* and *a′* chromosomes retain their looped forms until the other chromosomes have completely segmented, so that they evidently represent the open-ring type described by Farmer and Moore. Actually they become abbreviated, last of all being drawn out into rings by the method of longitudinal division described for their fellows. Thus



Four types of division exhibited by the heterotype chromosomes in metaphase.

I. Median fibre attachment—the first seven chromosomes divide in this manner.
II. Subterminal fibre attachment—four of the later bivalents to divide are of this type.
III and IV. The *a* and *a′* chromosomes which retain the looped condition of the bouquet threads till the other fourteen are already in telophase.

the segmentation of the ring-like heterotype chromosomes transversely is brought about by the separation of the diplotene threads longitudinally for all cases alike.

Great importance is attached to the retarded division of the *a* and *a'*, chromosomes, since their behaviour emphasises how easy it is to read the sequence of events in the heterotype metaphase in the wrong order unless the attempt is made to follow the genesis of each unit separately. No doubt it is for this reason that the erroneous telosynaptic description of the direct formation of rings from loops by transverse division of the diplotene threads has arisen.

Regarding the second maturation divisions of the male germ cells there is nothing to add to previous work, since it is agreed by all that the second division is equational. This does not necessarily imply that reduction is effected in the heterotype mitosis; for so far as the male is concerned there is no evidence that the line of separation of the diplotene components corresponds to the plane in which the leptotene filaments approximate in synapsis. It is believed, however, that this is definitely the case in the oocyte. The point is really very important; if, and only if, data respecting pre- or post-reduction are available, can the process of gametic segregation be demonstrated directly. On the evidence submitted it would appear: (*a*) that the filaments which conjugate side by side in the bouquet stage correspond to whole premeiotic chromosomes; (*b*) that the maturation divisions are of such a type as to provide for a segregation of alternate components of the bivalents so constituted. Respecting the further question of the relation of the heterotype chromosomes to the biparental complex of the zygote no data are submitted; and the reality of synapsis will not, therefore, be discussed. The conclusions here advocated are in harmony with those of other recent workers on Orthopteran chromosomes, namely: Wenrich, 1917 (22), Gérard (23), Robertson, 1915 (24), working on Acrididæ; Otte, 1907 (25) and Mohr, 1915 on the Locustidæ; Stevens, 1912 (26) on Centophilus; also Robertson on Tettigidæ and Vejdovsky, 1912 on locustids. Further it may be said that Baumgartner's figures of the metamorphosis of the heterotype chromosomes in Gryllus likewise suggest an original parasynaptic union (30).

With respect to the problem of synapsis in the Orthoptera, it may be said that no group has provoked more controversy; but while divergence exists to a very great extent, it must be remembered that the bulk of the work is concerned mainly with chromosomal individuality and the Accessories; few authors like Wenrich and the present writer have set out primarily to investigate the mode of synapsis. Hence the conclusions that have been expressed have been based too often on an inadequate supply of material showing the earlier stages; no data respecting these stages in the oocyte, where they are particularly clear; and, finally an incomplete series showing the critical stages in heterotype metaphase of preparations fixed with Flemming's reagent. An account of telosynapsis in the closely allied Plecoptera has recently been published by Nakahara, 1919 (28); the material was partly fixed in Bouin; it is implied that only adult testes were employed; and it is, therefore, not surprising that typical early stages are lacking, while the evidence regarding the first division is clearly fragmentary. For a full discussion of Orthopteran gametogenesis the reader is referred to the excellent review of the subject by McClung (29), who expresses no judgment on the original method by which homologous chromosomes are brought into association. Apart from the work of Nakahara, one other recent communication in part adheres to the telosynaptic interpretation of the Orthopteran tetrad. According to Payne, 1914 (33), some of the rings in Forficula are transformed in the manner described by Farmer and Moore, Sutton, Davis and other pioneer workers; while others conform to the mode described here as also by the list of authors quoted above. Such a view makes the earlier events of synapsis so unintelligible as to amount to a practical denial of synapsis. In the light of the data given above regarding the *a* and *a′* chromosomes, as well as for the consideration stated, it is hardly possible to accept Payne's conclusion unreservedly until confirmed.

Summary.

(1) Oogenesis and spermatogenesis in Periplaneta have been studied, the former for the first time; special attention has been paid to the events of the telophase in its bearing on the relation of the events of the meiotic phase in animals and plants respectively.

(2) The chromosomes do not divide in the premeiotic mitoses, as they pass to the polar ends of the spindle; in the telophase, they become attenuated before passing into the reticulate condition, but do not display any evidence of cleavage.

(3) The splitting of individual chromosomes begins in the prophase, the separation of the halves being a process of progressive differentiation; cleavage is completed before they adopt the equatorial position in the metaphase.

(4) Metaphase counts confirmed Morse's contention that there are two accessory chromosomes in the female cells and an unpaired heterochromosome in the male.

(5) Oogenesis in Periplaneta offers much more favourable conditions for a study of the earlier events of synapsis than spermatogenesis, but in essentials the processes are similar, with the exception of the fact that the

accessory chromosomes in the female behave in all respects like the autosomes.

(6) In its earliest stages, the heterotype prophase differs from the premeiotic reticulate nucleus in the organisation of the chromatin in the form of elongated, finely beaded filaments, forming an intricate tangle.

(7) The polarised leptotene threads of the early bouquet are present in the full diploid number.

(8) Parallel conjugation of leptotene threads takes place in the bouquet stage at the point of maximal contraction.

(9) The diplotene loops of the post-synaptic spireme become straightened and abbreviated, in anticipation of their transformation into heterotype chromosomes.

(10) In both cases two of the bivalents retain their looped condition till the transformation of the remainder has already made considerable progress, and it is suggested that the differential rate of metamorphosis of annular heterotype chromosomes has given rise to the appearance that the latter are formed through the union of the free ends of a diplotene loop.

(11) The heterotype chromosomes are formed by the opening out of the shortened diplotene rods along the line of cleavage, so as to assume eventually the form of stretched rings.

(12) From the fact that, in the oocyte, the splitting of the diplotene filaments appears to correspond with the plane in which conjugation takes place, it is inferred that segregation of homologous chromosomes is effected in the heterotype mitosis.

(13) A comparison has been made of the events of the meiotic phase in Periplaneta and Osmunda, as set forth in Miss Digby's most recent work; the existence of a single contraction phase in animals, and the character of the telophase in premeiotic divisions, indicate that there is no valid basis for the comparison of the initial processes in animals and plants.

(14) The elimination of "chromatin" particles in the diffuse stage of the oocyte nucleus has been fully investigated; it has been shown that the formation of yolk is consequent upon the elimination of material from the plasmosome, and the suggestion is made that similar nuclear particles in other animals arise from the plasmosome.

This research was conducted in Prof. MacBride's laboratory at the Imperial College of Science; acknowledgment is made to Profs. MacBride, Doncaster, and Dobell—to the former for his kindness in reading the MS., and to the two last named for bibliographical references.

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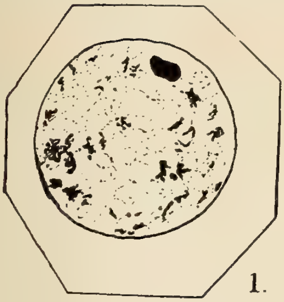

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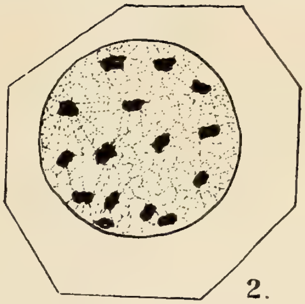

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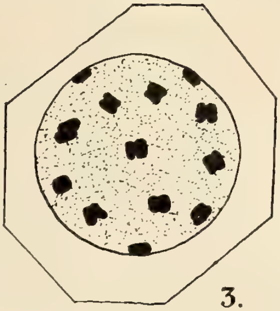

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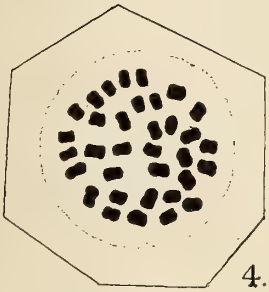

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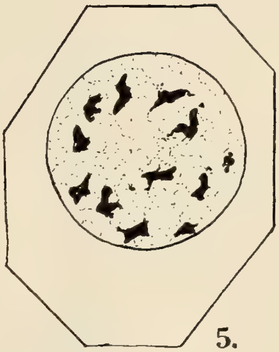

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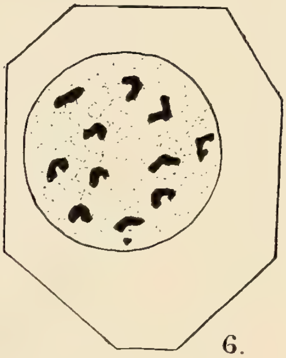

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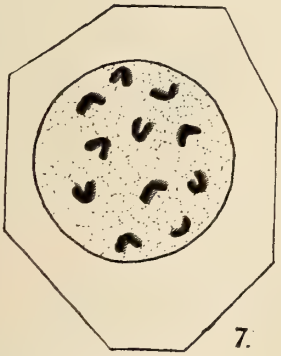

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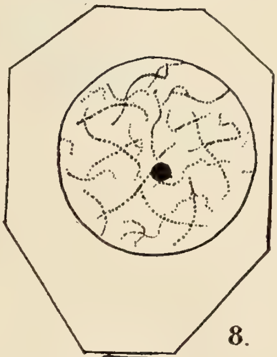

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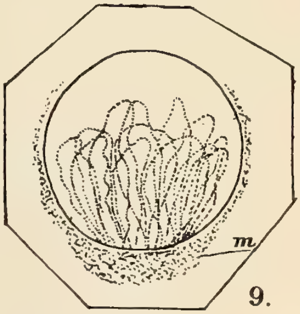

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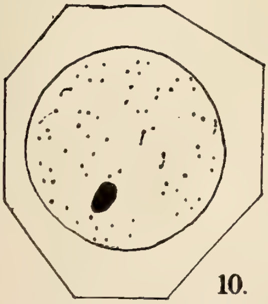

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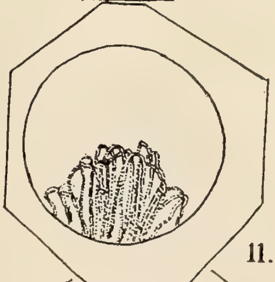

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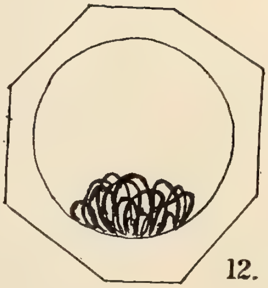

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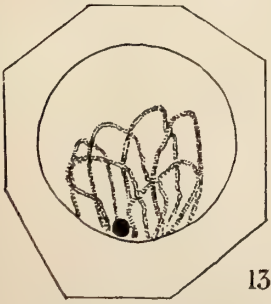

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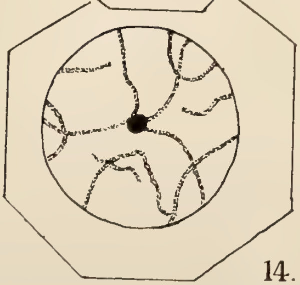

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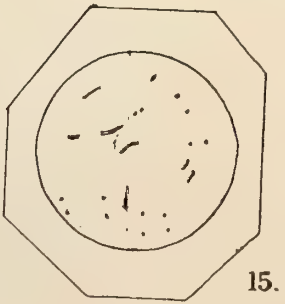

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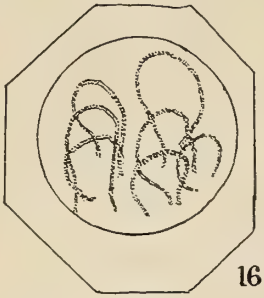

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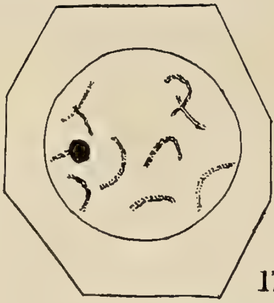

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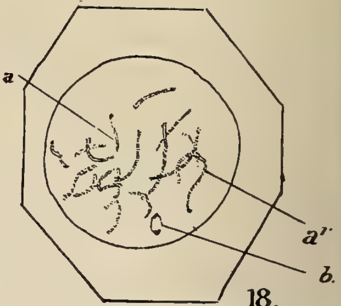

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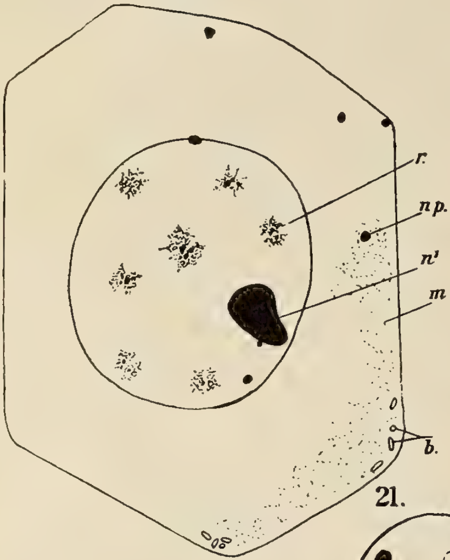

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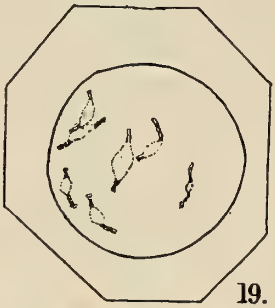

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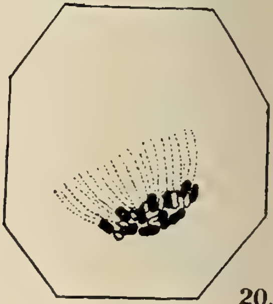

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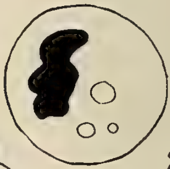

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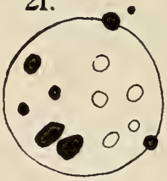

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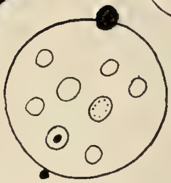

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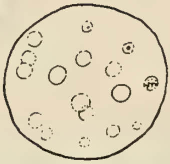

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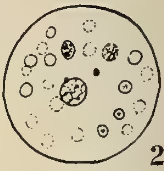

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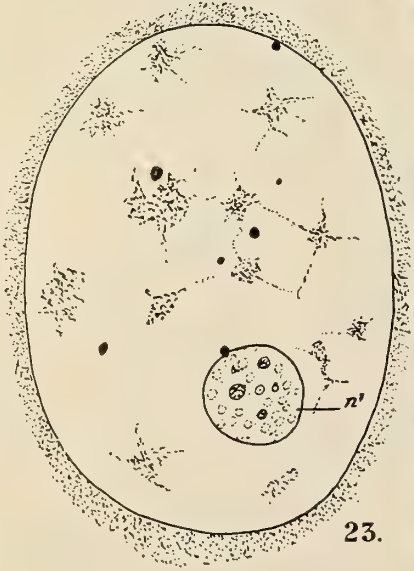

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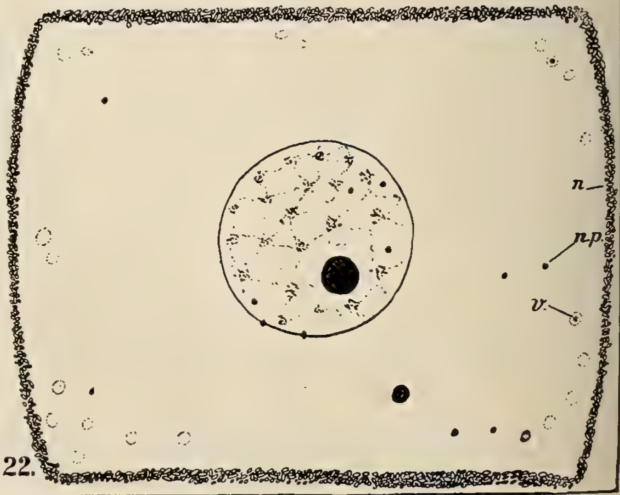

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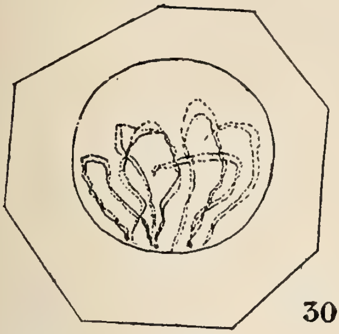

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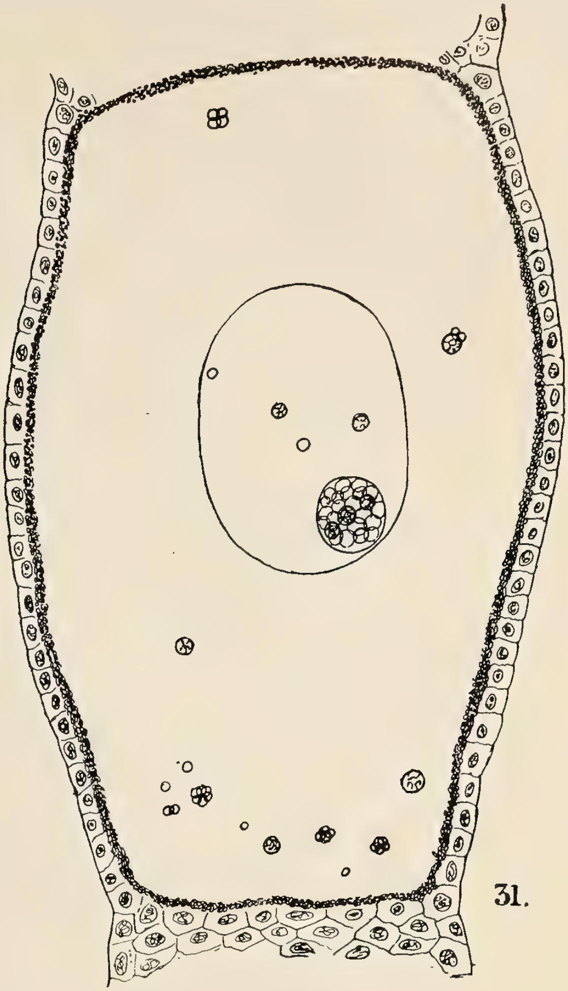

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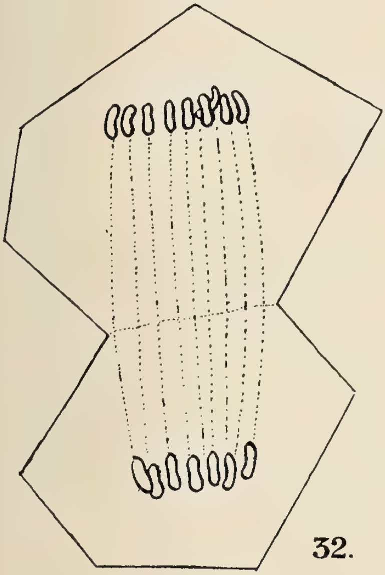

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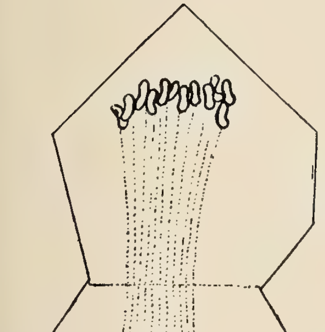

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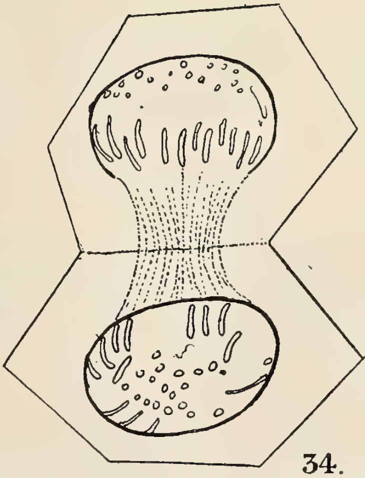

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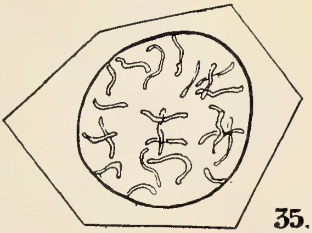

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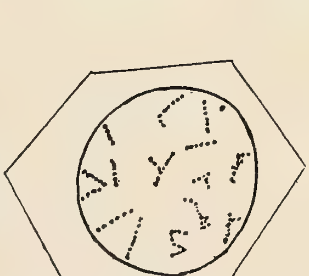

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DESCRIPTION OF PLATES.

Plate 10.

Figs. 1–3.—Oogonial prophase, stages.

Fig. 4.—Oogonial metaphase, equatorial plate (34 chromosomes).

Figs. 5–7.—Follicular prophases.

Fig. 8.—Early leptotene.

Fig. 9.—Leptotene bouquet. (Lateral view.) *m.*, mitochondria.

Fig. 10.—Ditto in section.

Fig. 11.—Synaptene stage.

Fig. 12.—Pachytene stage (maximal contraction figure).

Figs. 13, 14.—Diplotene post-synaptic bouquet.

Fig. 15.—Early diplotene stage in section.

Fig. 16.—Segmentation of the postsynaptic spireme.

Plate 11.

Figs. 17–19 and 21.—Formation of and dissolution of the tetrads.

Fig. 20.—Late oogonial anaphase.

Figs. 21, 22.—Very young oocytes at inception of growth phase. *n.p.*, nuclear particles; *m.*, mitochondria; *b.* and *z.b.*, Bacillus cuenoti; *v.*, cytoplasmic vacuoles.

Fig. 23.—Germinal vesicle of egg at later stage, showing the vacuolation of the plasmosome.

Figs. 24–29.—Stages in the appearance of the deutosomes within plasmosome in acid-fixed logwood preparations.

Fig. 30.—Zygotene stage of the bouquet in young oocyte.

Plate 12.

Fig. 31.—Egg at inception of yolk formation, showing migration of intranucleolar deutosomes to the periphery of the egg to form the deutoplasmic spheres.

Figs. 32, 33.—Spermatogonial anaphases.

Figs. 34–36.—Stages in the passage of the telophasic chromosomes into the resting nucleus reticulum.

On some Rostro-carinate Flint Implements and Allied Forms.

By Sir E. Ray Lankester, K.C.B., F.R.S.

(Received May 31, 1920.)

The specific type of flint implement which was discovered by Mr. Reid Moir, of Ipswich, in 1909, and described by me* as the "rostro-carinate" type, has been found and recognised by various observers since that date. The importance of these implements arises from two facts. The first is that they exhibit a design, or "sculptural form," distinct from that of the previously known palæolithic flint implements. They do not belong to the platessiform tongue-shaped Chellean and Acheuillian types, nor to the amygdaloid somewhat smaller type of the same age. They cannot be grouped with the Moustierian pointed flakes and the numerous scrapers, knives and borers of various Palæolithic ages; nor can they be referred to any recognised Neolithic type of implement.

The second fact is that several specimens of this novel type—the rostro-carinate—have been found in the detritus-bed (bone-bed) of Suffolk, underlying the Red Crag, and in the remarkable "stone-bed" or "flint-bed" underlying the Norwich Crag in Norfolk. In those deposits flint implements of the large types familiar in the terrace gravels of our river valleys are unknown.

Many "rostro-carinates" of well-defined character have, since my original description of the type, been found, some in deposits of later date than the sub-Crag detritus-bed of Suffolk and the sub-Crag stone-bed of the Norwich area, others actually in the detritus-bed and stone-bed. Mr. Reid Moir has obtained typical specimens from the Middle Glacial Gravel and from the Chalky Boulder Clay near Ipswich. Prof. Marr has found one in a gravel of the Chellean age† near Cambridge. Several have been found in a remarkable deposit, described as "a raised beach," at the Island MacGee, near Larne (Belfast), of which I have figured the most remarkable specimen in my memoir on the Norwich test specimen.‡ Mr. Reid Moir has also recently published an account§ of a noteworthy series of specimens from river-terrace gravels, which exhibit transition, in form and sculptural treatment, between the typical "rostro-carinate" and the large platessiform tongue-like implements of Chellean age.

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* 'Phil. Trans.,' B, vol. 202, pp. 283–336 (1912).

† Prof. Marr obtained this implement at the Traveller's Rest Pit, Huntingdon Road, Cambridge. He writes that "the gravel there contains only worn Chellean and Acheulean implements below, and either latest Acheulean or earliest Le Moustier (or both) above. The latest things are not much worn." None of it is pre-Chellean, but this does not exclude the possibility of the inclusion of a few pre-Chellean derivatives.

‡ 'Occasional Papers,' No. 4, Royal Anthropological Institute, 1914, text-figs. 9–14.

§ 'Phil. Trans.,' B, vol. 209 (pub. 1920, received 1917).

It is impossible to assign any of these later "rostro-carinate" specimens to the geological horizon or deposit corresponding to the date or period at which they were fabricated by man. Some have been found in such positions and such beds that it is *possible* that they were manufactured at the same date or period as were those found beneath the Red Crag and the Norwich Crag.

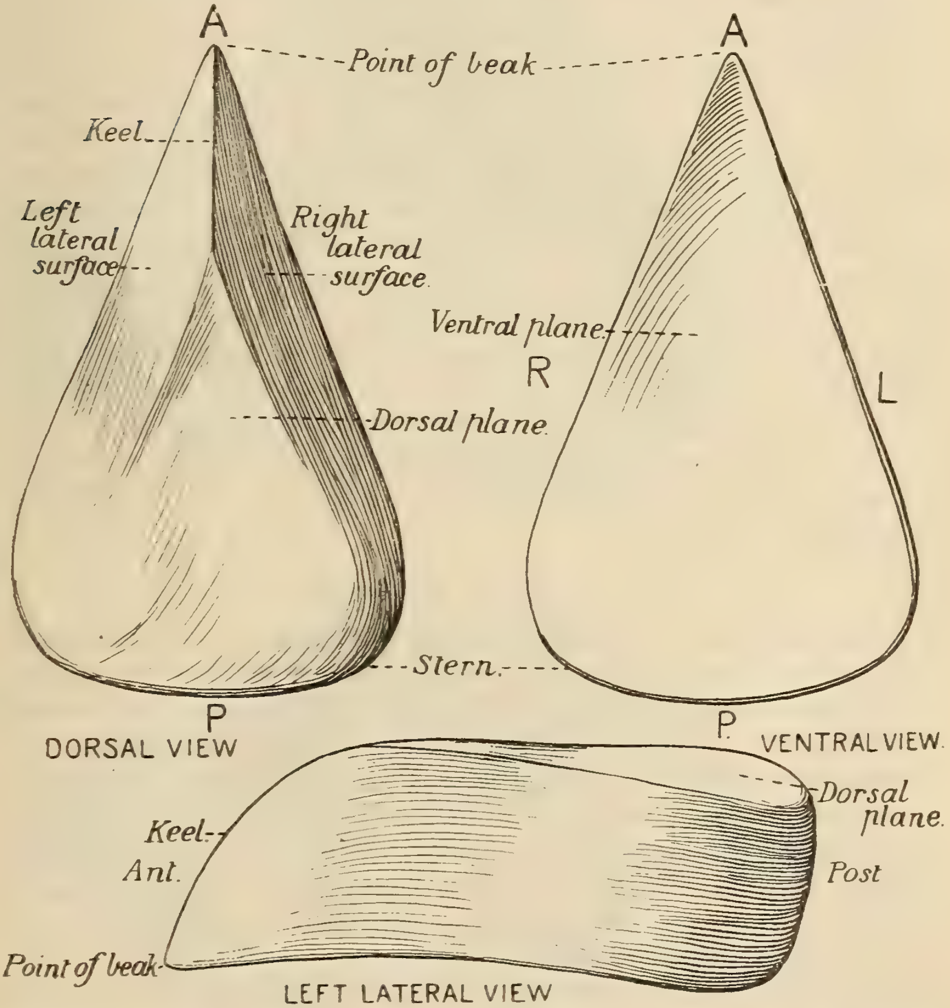
A
Point of beak
A
Keel
Left lateral surface
Right lateral surface
Ventral plane
R
L
Dorsal plane
Stern
P
DORSAL VIEW
P
VENTRAL VIEW
Dorsal plane
Keel
Ant.
Post
Point of beak
LEFT LATERAL VIEW

Fig. 1 (From the 'Phil. Trans.,' B, May, 1912).—Diagrams showing the ideal form aimed at by the makers of the rostro-carinate flint implements or "Eagles' Beaks." A, anterior; P, posterior; R, right; L, left. (In the present memoir I use the term "carina" in place of "keel," and sometimes refer to the "dorsal plane" as the "dorsal platform.")

They *may* have remained undisturbed on the land surface, or embedded superficially, for long ages after that period, and may have been swept up by the waters which deposited a series of later gravels. Some of them exhibit such evidence of water-wearing, since they were flaked into shape, as to *suggest* a more ancient origin and history than that of palæoliths of recognised types found in gravels of the age in which these specimens occur. But this is not, by any means, invariably the case. It is true of Prof. Marr's Cambridge specimen (and of others obtained by Mr. Reid Moir at Mundesley, not yet published), but it is not the case of others which, nevertheless, have been taken from gravels deposited at a period long subsequent to the sub-Crag detritus and stone-bed. The rostro-carinate implement from the raised beach at Island MacGee, figured by me in the Royal Anthropol. Inst. 'Occasional Papers,' No. 4, is a case in point. It has suffered very little from water wear or attrition. Remarkable in this respect is the specimen here figured (figs. 2 to 5) from Icklingham, the fracture edges of which are very clean and sharp, though not to the same degree as those of a newly fractured flint. Less fresh in appearance, and strongly iron-stained and polished, but still not much "rubbed down," are the rostro-carinates figured in this paper from the Oise (figs. 6, 8, 10, 12), from Burnham Beeches (figs. 7, 9, 11), and from Sonning (figs. 13 and 14).

Nomenclature of Rostro-carinates.

It is often convenient to refer by means of *names* to the more remarkable specimens of rostro-carinate flints which have been described and carefully illustrated in published memoirs. Thus we have the "Evans-Lackenheath specimen,"* the "Moir-Foxhall pebble,"† the Moir-Whitton‡ Uncinate,"§ the "Moir-Whitton sub-Crag massive"|| being a few from among those figured and described in my memoir in 1911. Then we have the "Clarke-Norwich test specimen"¶ and the "Lankester-MacGee specimen,"** so named to distinguish it among the large series collected by Mr. W. G. Knowles, of Ballymena. The list could be advantageously extended by giving like names to the more important mid-glacial and Boulder Clay specimens figured by Mr. Moir, whilst the transitional series figured and described by him†† are

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* 'Phil. Trans.,' B, No. 290, figs. 4 and 5.

† 'Phil. Trans.,' B, No. 290, figs. 2 and 3.

‡ Whitton is the name of the parish in which the large "brick-pit" of Messrs. Bolton and Laughlin is situated on the border of the town of Ipswich.

§ 'Phil. Trans.,' B, No. 290, figs. 6 and 7.

|| 'Phil. Trans.,' figs. 18 and 19, and Plate 17.

¶ 'Occasional Papers,' No. 4, Royal Anthropological Institute, Plates 1, 2, and 3.

** Text-figs. 9–14 in the same memoir.

†† 'Phil. Trans.,' B, No. 367 (1920).

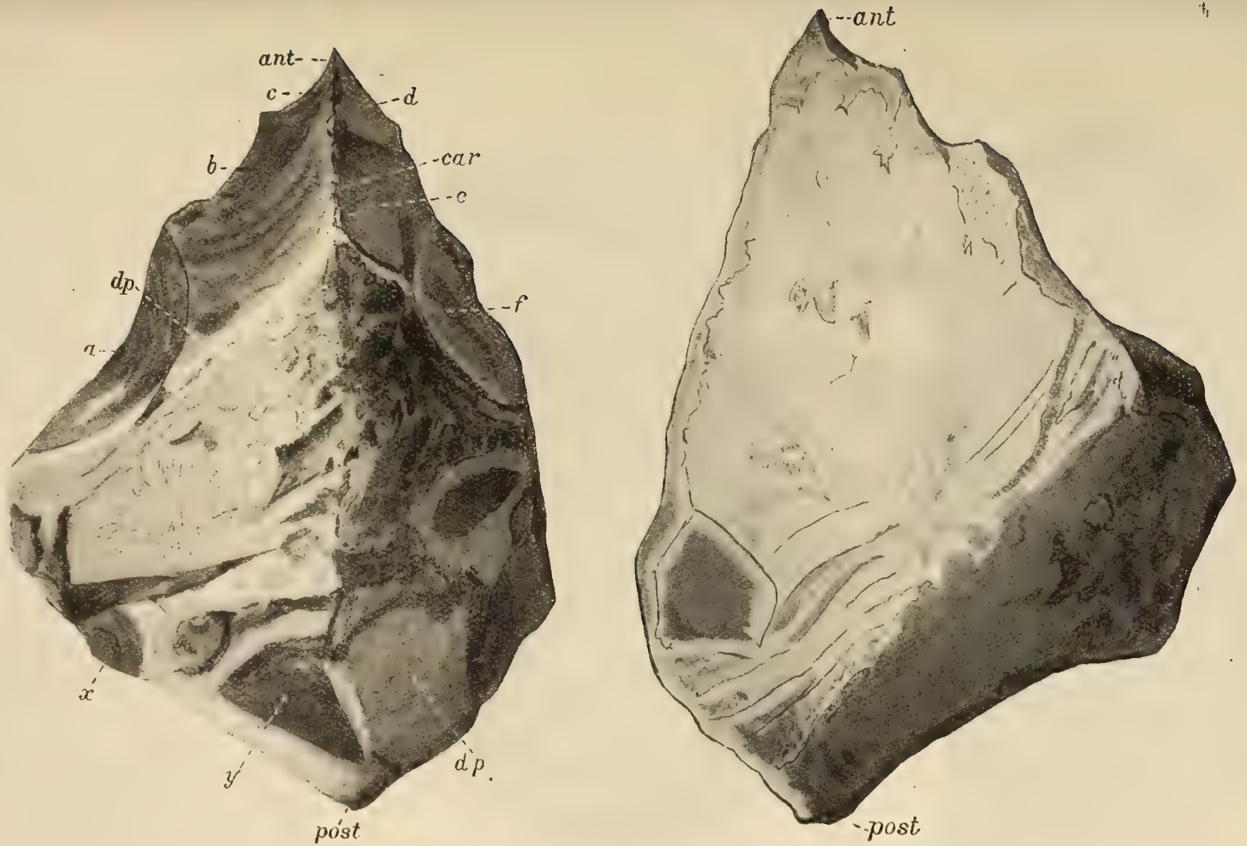

FIG. 2.

FIG. 3.

FIGS. 2 and 3.—Dorsal and ventral aspect of the Sturge-Icklingham rostro-carinate (drawn of the actual size). *Ant.*, anterior point ; *post.* posterior region ; *car.*, carina ; *d.p.*, the dorsal plane ; *y*, site whence a "trimming" has been removed ; *x*, a similar site ; *a*, *b*, *c*, the concavities left by the removal of the three chief "flakings" of the left lateral region of the rostrum ; *d*, *e*, *f*, the concavities left by the three chief "flakings" of the right side. Note the unworn condition of the carina and the unsymmetrical shaping of the mass or butt of the flint behind the rostrum.

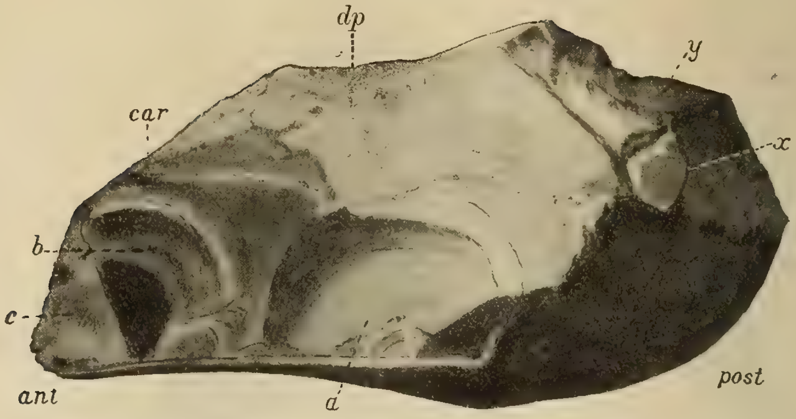

Fig. 4.

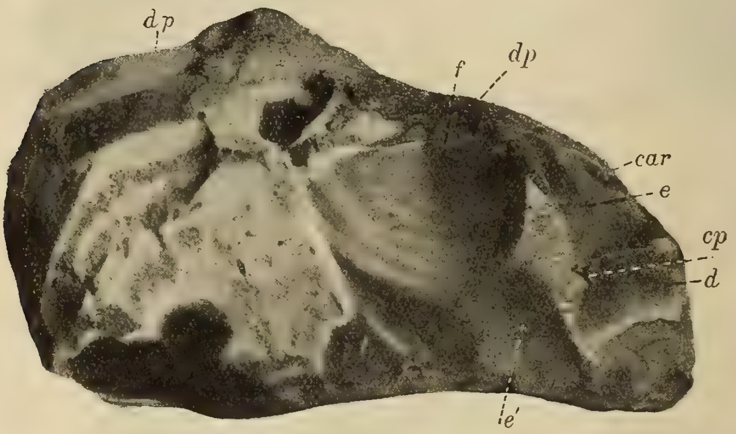

Fig. 5.

Figs. 4 and 5.—Left lateral aspect and right lateral aspect of the Sturge-Icklingham rostro-carinate. The close similarity of the outline to that of the similar lateral view (whether right or left) of the "Norwich test specimen" should be verified by comparing these two figures with the figures of the Norwich specimen on the opposite page. The lettering here is the same as in figs. 3 and 4, except *cp* in fig. 5, which points to the "centre of percussion" of the blow by which the flaked surface *e e'* was formed. The formation of the concave flaked surface *f*, fig. 5, by a blow downward from the dorsal plane, is well shown by its rippling.

Fig. 4 is from a wash-drawing; fig. 5 is a reproduction of a photograph of the actual specimen.

readily distinguishable as the "Kendall-Savernake" transitionals I and II, the "Allen-Brown-Dawley" transitional, the "Smith-Warren-Hill," the "Greenhill-Clapton," the "Moir-Ipswich," and the "Barnes-Axminster" transitionals.

Those specimens of rostro-carinate which are here figured for the first

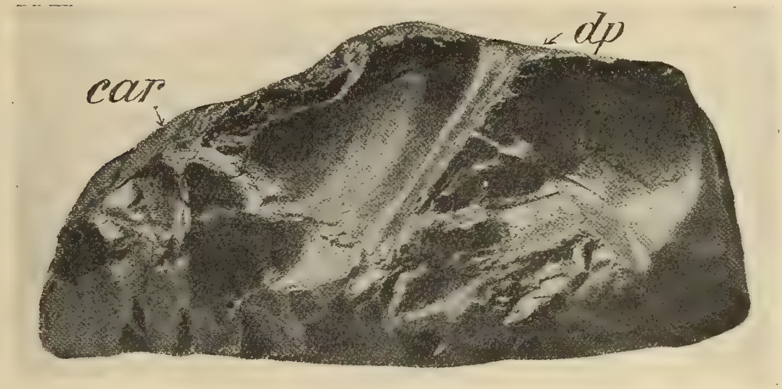

FIG. 4 *bis*.

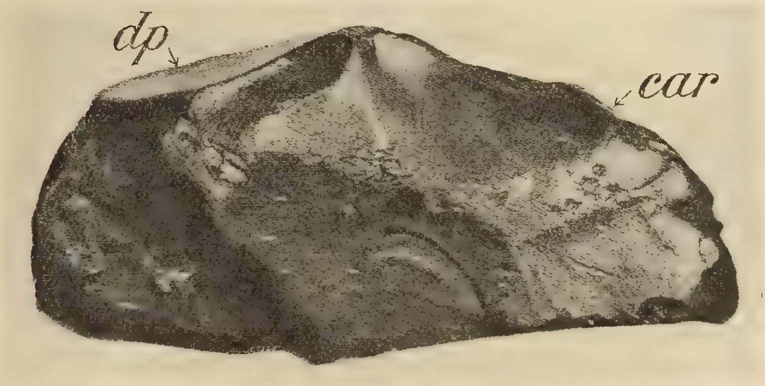

FIG. 5 *bis*.

FIGS. 4 *bis* and 5 *bis*.—Right and left lateral aspect of the Norwich test specimen (published by me in the 'Occasional Papers,' No. 4 (1914), "Roy. Anthrop. Institute"), to compare with the similar views of the Sturge-Icklingham specimen.

time I propose to name as follows :—The Sturge-Icklingham rostro-carinate the Capitan-Oise and the Rowell-Burnham uncinates; the Peake-Sonning uncinate; and the Moir-Martlesham jack-plane.

Section I.—*The Sturge-Icklingham Rostro-carinate.*

This implement was picked up in a field at Icklingham, Suffolk, and given to Mr. Moir by Dr. Allen Sturge. It is now in the Ethnological Department, British Museum, Bloomsbury. The edges of its fractures are not absolutely fresh and sharp, but are very little blunted. It is not iron-stained; its unfractured surface is white, its fractured areas are black and translucent at the edges. It is in a mineral condition similar to that of hundreds of other flints found in that district.* Four views are given in figs. 2, 3, 4, and 5 of the actual size of the specimen. I also reproduce in fig. 1 the diagram of the ideal rostro-carinate published by me.†

In general sculpture the Icklingham specimen conforms very closely to the ideal diagram, but is not trimmed to a symmetrical oval shape posteriorly. As seen from above (what I conventionally call "the dorsal aspect"), the mass on the left side of the "carina" and middle line is seen to be the larger and to project asymmetrically as compared with the right side. The maker of the implement attached no importance to this defect in symmetry, and did not risk any further trimming of the piece to bring it into a more regular shape.

This implement has obviously been fashioned from a piece of flint broken in the first place to a tabular form with ventral plane surface and dorsal plane surface. The dorsal surface consists in part of the original cortex of the flint nodule. It is of the usual opaque white finely granular texture. The whole of the ventral surface and of the posterior more or less vertical surface (*d.p.*, fig. 2) has been produced by ancient fracture (preceding the flaking which has formed the sides and the carina of the implement), and is white ("decomposed" or "*re*-composed" flint), but this white layer does not extend so deeply into the black flint as does the more primitive white area of cortex. It is a mere skin of 1/40 of an inch thick, whereas the primitive cortex is 1/10 to 1/5 of an inch thick. The latter has a pale brownish tint as compared with the former, which is of a purer white. The original cortex is finely granular in texture, whereas the later white skin is smooth and enamel-like, and tends to flake off in thin superficial laminæ (as often seen in "weathered" flints).

The man who fashioned the flint to its present rostro-carinate shape obtained it as an irregular block of superficially whitened or decomposed flint, about an inch and a half to 2 inches thick, with a flat upper and lower surface, having an area of some 4 inches by 3 inches. He struck off very few, yet dexterously taken, flakes which exposed the black unchanged flint (fig. 2 *a*, *b*, *c* left, and *d*, *e*, *f* right). Thus were shaped the two sides of the implement, converging to a median point or rostrum (fig. 2, *ant.*) and separated by a sharp-edged "carina," like the keel of a boat's bow (p. 331 and figs. 3 and 4, *car.*). The three principal flakes on the left lateral face, lettered *a*, *b*, *c* in fig. 2 and fig. 4, have all been struck from the ventral plane upwards. The most anterior is much smaller than the other two, but the fracture-ripple-marks in each case demonstrate the site and direction of the blow which produced them. On the right side a large flake has been removed from the surface marked *f*, the ripples on which show that the blow was delivered downwards from the edge of the dorsal platform above it ; *d* marks a smaller area cleared by an upward blow, probably after the area marked *e*, which extends round *d* to the edge of the ventral plane, had been formed. The area, of which the upper half is marked *e*, was formed by a blow, shown by the ripple marks to have been applied vertically to the surface near the point where in fig. 5 (which is a photograph) a ⋈-shaped mark is obvious. A small centre of percussion *c.p.* is visible in the specimen just below this mark. Some five small black areas (some unlettered, but two marked *x* and *y*, in figs. 2 and 4) scattered on the otherwise white hinder region of the implement, show where "trimming" fractures were administered; they have no influence on the important part of the implement—the rostral area—which, with its deftly shaped black sides and sharp clean-cut carina, has been struck into the desired form by three flakings on each side of the mid-line, only one of which was struck downwards from the dorsal plane or platform.

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* Mr. Moir writes: "This rostro may be late Palæolithic or even Neolithic; on the other hand, the Icklingham fields are strewn with undoubted glacial material, and this particular flint may have been derived from some pre-existing glacial deposit." In the present state of knowledge it seems impossible to give a definite opinion as to its age.

† 'Phil. Trans.,' B, No. 290.

In most rostro-carinate implements, the flat ventral surface itself, its sharp lateral edges where the flaked sides cut it, and the acute point in which it terminates anteriorly, are what appear to have been *useful* features—the first for flattening and smoothing; the second for cutting, planing, and scraping; the third for pushing and boring wedge-wise into tough material. Usually the carina (see diagram, fig. 1, and *car.* in figs. 2, 4, and 5) is so much blunted and splintered as to be apparently of no value as a "cutting edge." This bluntness may, however, as Mr. Reid Moir thinks, be due to the "wear and use" of the implement, and the carina or keel may usually have been, when the implement was newly produced, a valuable cutting edge. In any case this is certainly true of the Sturge Icklingham specimen here figured, which has the best preserved edge to its carina of any rostro-carinate known to me.

Section II.—*The Moir-Whitton Yellow Mid-glacial No. II.*

This (figs. 5*a*, 5*b*, and 5*c*) is an interesting and valuable specimen, because it is a thoroughly typical rostro-carinate with dorsal plane (fig. 5*b* D.P.), well marked ventral plane occupying the whole ventral area (fig. 5*c*), good carina (*car.*) and distinctly *uncinate* rostrum, projecting somewhat below the ventral plane.

The specimen is of a uniform yellowish-brown colour, glistening, that is

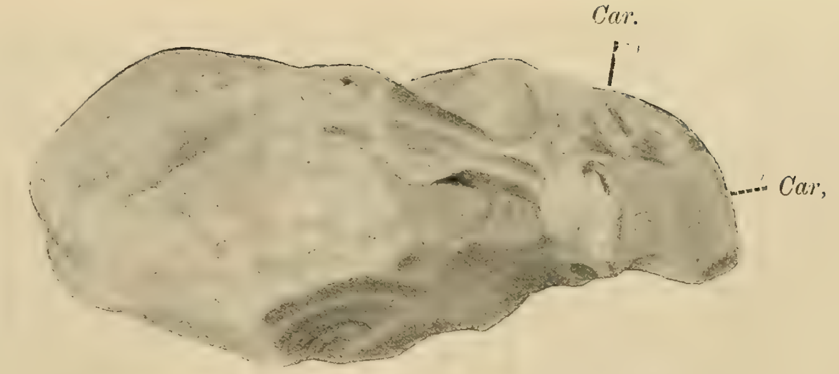

FIG. 5*a*.

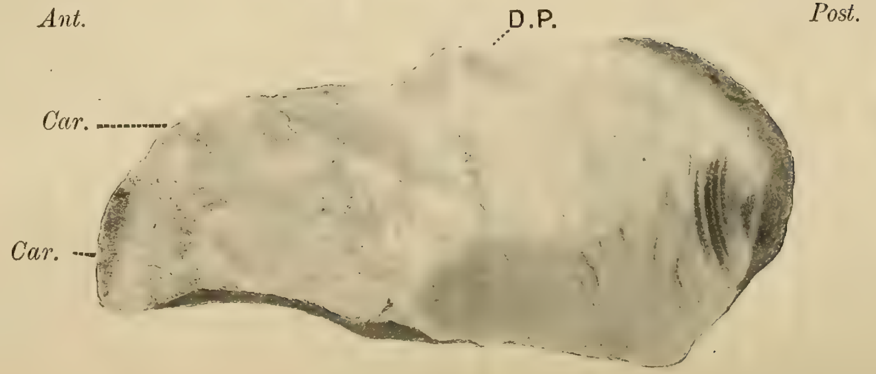

FIG. 5*b*.

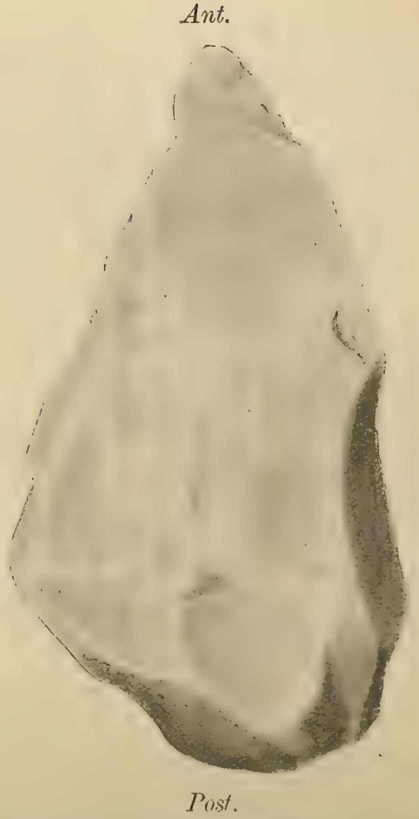

FIG. 5*c*.

FIGS. 5*a*, 5*b*, and 5*c*.—Drawings of the "Moir-Whitton yellow mid-glacial" rostro-carinate, now in the Ipswich Museum. It was figured by Mr. Reid Moir in 'Journ. Roy. Anthrop. Institute,' vol. 46, p. 204. Fig. 5*a*.—View of right lateral region. Fig. 5*b*.—View of left lateral region. Fig. 5*c*.—View of the ventral plane. Letters: *Ant.*, anterior; *Post.*, posterior; *Car.*, carina; *D.P.*, dorsal plane or platform. Drawn of the actual size.

to say, slightly sand-polished. It was obtained by Mr. Moir from Bolton and Laughlin's great pit at Whitton, Ipswich. It is less sharp in its edges and more "polished" than is the Sturge Icklingham specimen just described. It has also special importance as coming from the Mid-Glacial Gravel. Other implements have been described by Mr. Moir from this locally well-developed gravel, as well as the present specimen.

Section III.—*The "Capitan-Oise" and the "Rowell-Burnham" Uncinates.*

The remarkable coincidence in the sculpture of these two implements renders it desirable to describe them together. The Capitan-Oise specimen (figs. 6, 8, 10, and 12) was sent to me by Prof. Capitan, of Paris, in 1915, as being, in his opinion, an implement of the rostro-carinate type. He writes: "Il provient des graviers du fond de la vallée de l'Oise, sans provenance précise, mais certainement de la basse vallée. Ces graviers débutent (comme faune et industrie) au Chelléen et vont jusqu'au Moustérien."

When this specimen reached me, I was already in possession of that found in the gravel at Burnham Beeches (Thames valley) by Dr. Rowell, and the close similarity of the two specimens was at once obvious. In both, the anterior region or "rostrum" has a somewhat "hooked" or claw-like appearance, which may be described as "uncinate." This shape of rostrum was already known to me in some other rostro-carinates, for instance, in the "Moir Ipswich uncinate," drawn in figs. 6 and 7 of my memoir.* Further, both the Oise and the Burnham Beeches specimens present none of the original cortex, but are flaked all over, excepting a small area of the Oise specimen, shown in fig. 8, in deep shadow below the line *llv.* (which I identify as corresponding to the left latero-ventral margin of the ideal type, fig. 1), and they are iron-stained all over—the Oise specimen more deeply than the other.

But the two most important features shown by the Oise and Burnham Beeches specimens are, *first*, the very large and flat dorsal platform or plane, marked D.P. in the figures, showing in each case the curved ridge-like ripple marks produced by the single blow by which it was formed, and *second*, the apparent absence of anything like the great "ventral plane" of the ideal type, which in most rostro-carinates is a very obvious and important feature of their architecture. In both specimens a keel-like ridge (*rlv.* in the figures) forms the ventral boundary of the rostral region of the implement, though the posterior half is broad and butt-like. We

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* 'Phil. Trans.,' B, No. 290.

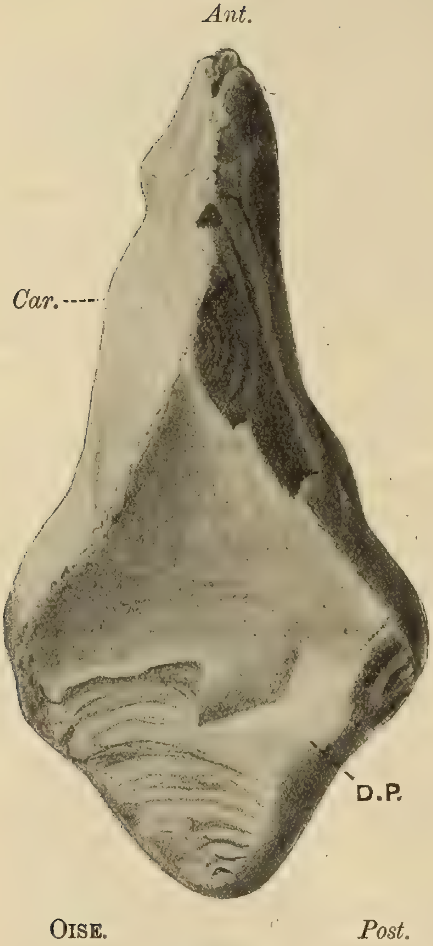
Ant.
Car.
D.P.
OISE.
Post.

FIG. 6.

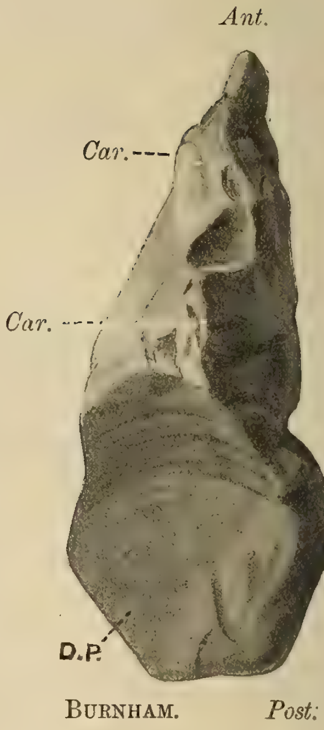
Ant.
Car.
Car.
D.P.
BURNHAM.
Post.

FIG. 7.

The "Capitan-Oise" and "Rowell-Burnham" uncinates.

FIG. 6.—Dorsal aspect of the Capitan-Oise specimen.

FIG. 7.—Similar view of the Rowell-Burnham specimen.

have in this case a disappearance or "transference" of the ventral plane, similar to that described by Mr. Moir in some of his transitional specimens.*

Mr. Moir supposes that, in his transitional series, the ventral plane of a possible rostro-carinate has been chipped away by flaking from *both* sides, so as to convert it into a cutting ridge, forming one side of the completed platessiform implement.

That may be so in some instances. We must not expect that the successive steps in human workmanship, aiming at the realisation of a desired form—as, for instance, in the supersession of the rostro-carinate by the platessiform type—will be invariably the same as though they were controlled by a morphological inherent quality. Primitive man succeeded in producing a useful flattened leaf-shaped implement, in some cases, by symmetrical trimming down of the dorso-lateral mass of a possible rostro-carinate, leaving the ventral plane intact. This Mr. Moir has recognised, and applied to it the name suggested by me, viz., "batiform." Further, it appears to me that the early flint-workers, when not desiring to combine the "planing" and flattening quality of the typical rostro-carinate with those of its point and carina, often got rid of the "ventral plane," *not* by first making it and then chipping it away on both sides, but by so chipping the flint, from the first, as to *dislocate* or "transpose" the "ventral plane," and throw it up, as it were, to form part of either the right or the left lateral areas.

This, it appears to me, is what has been done in the two uncinate rostro-carinates from the Oise and from Burnham Beeches. What would have been developed as a flat ventral plane parallel to the dorsal plane in the making of a typical rostro-carinate is, in both of these specimens, given an upward tilt and allowed to "run into" (or one may say is "flaked into") the left lateral region. Probably there was no attempt from the first handling of the flint at the production of a ventral plane. After all, this is, perhaps, a mere question of descriptive terms. I doubt whether it is possible to interpret the operation of man's ingenuity in manufacture—even that of Palæolithic man—by laws suggested by the morphology of living organisms. The main point is that we have evidence of what blows were struck, and what shapes were thereby produced—and we employ comparisons with organic shapes to describe, but not to explain, them.

If we had any real knowledge of the mechanical needs and urgent requirements of primitive man, as well as of the habits and opportunities of those whose work we discover, we might, instead of vague suppositions, offer plausible suggestions as to the value to him of the shapes of those implements of his which we dig up. I am strongly of opinion that the very large

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* 'Phil. Trans.,' B, 1920.

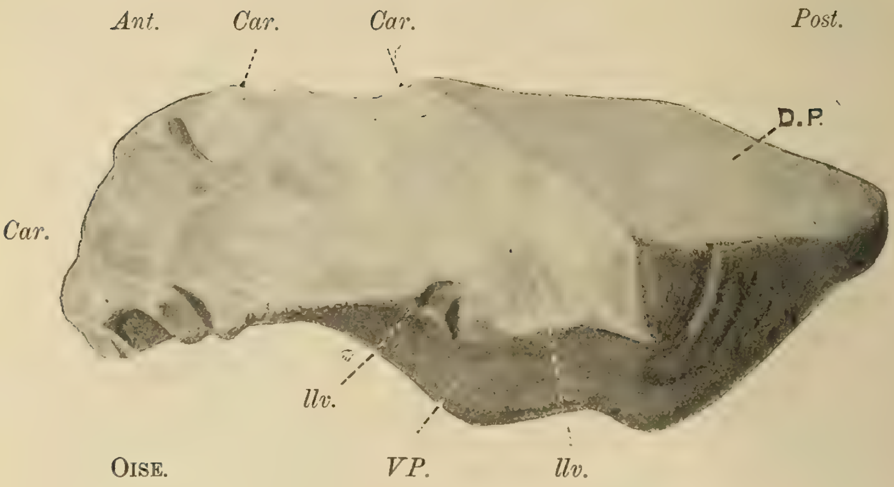

Ant.
Car.
Car.
Post.
D.P.
Car.
llv.
VP.
llv.
OISE.

FIG. 8.

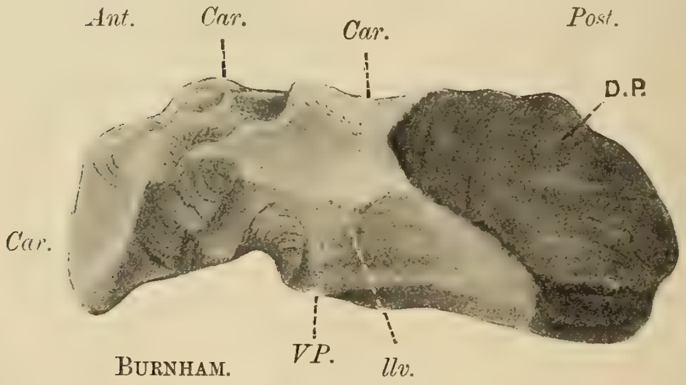
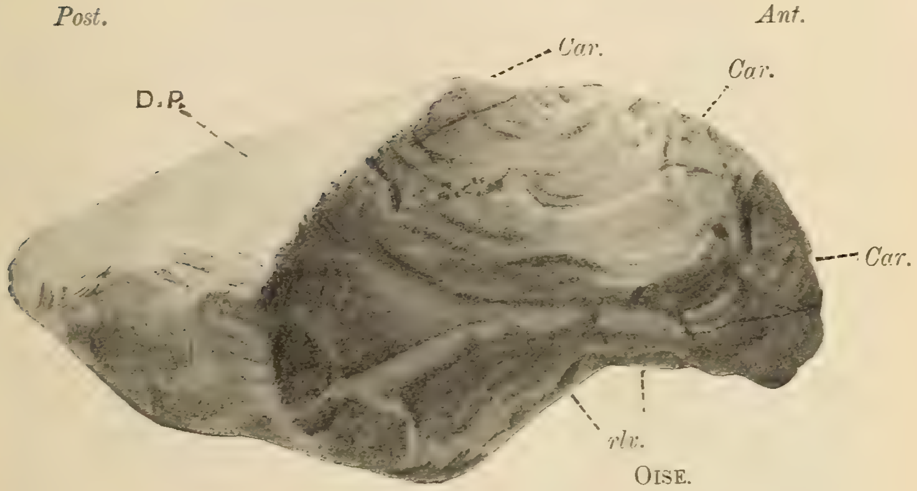

Ant.
Car.
Car.
Post.
D.P.
Car.
VP.
llv.
BURNHAM.

FIG. 9.

The "Capitan-Oise" and "Rowell-Burnham" Uncinates.

FIG. 8.—Left lateral aspect of the Capitan-Oise specimen.

FIG. 9.—Similar view of the Rowell-Burnham specimen. Lettering: *Ant.*, anterior; *Post.*, posterior; *D.P.*, dorsal plane or platform; *Car.*, carina; *llv.*, LEFT latero-ventral *arrête* or ridge separating the left lateral surface from the reduced and up-turned ventral plane; *VP.*, the obliquely up-thrown ventral plane (see fig. 12).


Post.
Ant.
Car.
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rlv.
OISE.

FIG. 10.

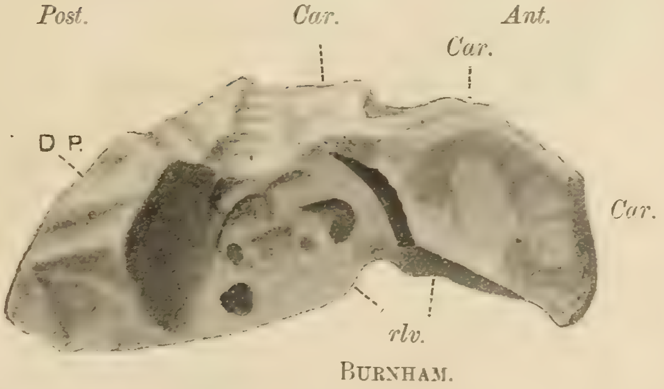
Post.
Car.
Ant.
Car.
D P.
Car.
rlv.
BURNHAM.

FIG. 11.

The "Capitan-Oise" and "Rowell-Burnham" Uncinates.

FIG. 10.—Right lateral aspect of the Capitan-Oise specimen.

FIG. 11.—Similar view of the Rowell-Burnham specimen. Lettering as in Figs. 6-9, excepting *rlv.*, the RIGHT latero-ventral *arrête* or ridge separating the right lateral surface from the reduced and up-turned ventral plane (see fig. 8).

dorsal plane or platform, D.P. in the Oise and Burnham specimens, must have served a special purpose. I suggest that it would facilitate the binding of the implement to a short wooden "hefting piece" which (as we see in Neolithic and modern Melanesian examples) was inserted at right angles into a stout handle. Of course, in place of a hefting piece, the ancient men may have employed a naturally bifurcate vegetable stem, as do some of the Melanesians of the present day.

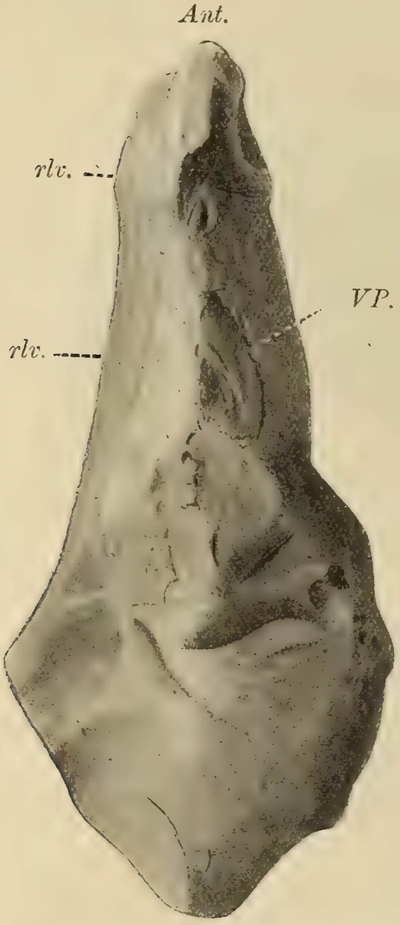

Fig. 12.—Anti-dorsal aspect of the Capitan-Oise specimen, showing the median position taken by the right latero-ventral *arrête* or ridge, *rlv.*, and the upthrow of the ventral plane, *VP.*, to the left lateral face in the rostral region, whilst the broad "butt" or "stern" is treated merely as a massive base to the large dorsal plane.

Section IV.—*The Peake-Sonning Uncinate.*

The specimen drawn in figs. 13 and 14 was obtained in the gravel at Sonning (on Thames) by Dr. A. E. Peake, of Burford, and has been kindly lent by him to me for description. It is a large example of a form which may be considered as a derivative of the last two described, in which not only the "ventral plane," but also the dorsal platform or plane has been abandoned as an element in the modelling of the completed implement. It is simply a "rostro-carinate," reduced to a more or less "uncinate" rostrum, and a simple flaked "body" suitable for the hand-grip. Smaller flints, having the same general shape, are known, but the evidence of human workmanship in this larger specimen is more convincing than in others which probably are to be interpreted also as human productions. The series of parallel flakings on the left face of the Sonning specimen (fig. 13) are clearly of human origin. A large area of original cortex (x and y in figs. 13 and 14) is retained and forms a sort of collar, marking off the "rostrum" from the rest of the flint. In the explanation of figs. 13 and 14 I have suggested the possible identification of certain

FIGS. 13 and 14.—Left (fig. 13) and right (fig. 14) face of "the Peake-Sonning uncinate," drawn of the actual size. Letterings *x* and *y* point to a large area of unflaked cortex, the natural shape of which as a sort of trough has been used by the tool-maker to emphasise the overhanging rostrum (see *x*, *y* in fig. 14); *xp*, flaked edge of cortex; *car.*, carina; *car.**, identical point in figs. 13 and 14; *dp*, possible remnant of a dorsal plane; *vp*, probable representative of the ventral plane, now merged in the right lateral area; *n*, probable right latero-ventral *arrête*; *car.*, carina; *fl*, large flake-area on left side of rostrum; *xc* and *z*, identical points shown in the two drawings; *h*, a natural hollow in the flint, due to enclosure of organic remains (probably a sponge).

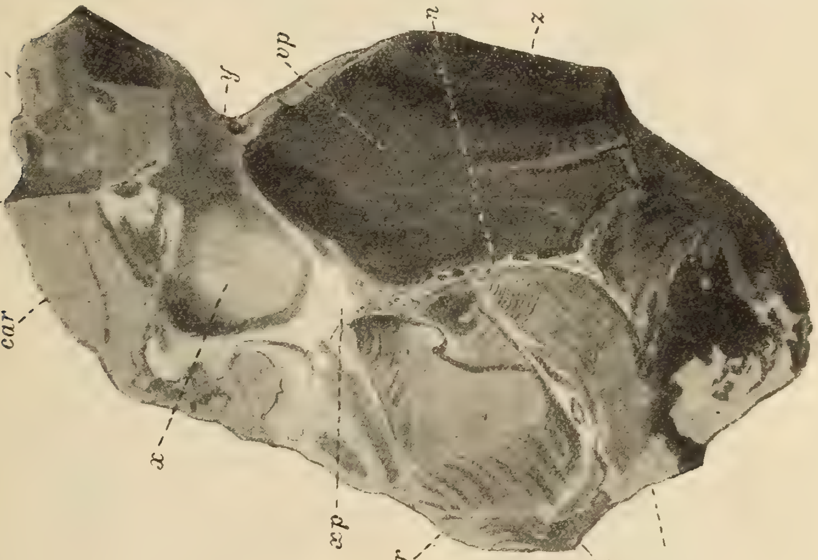

FIG. 14.

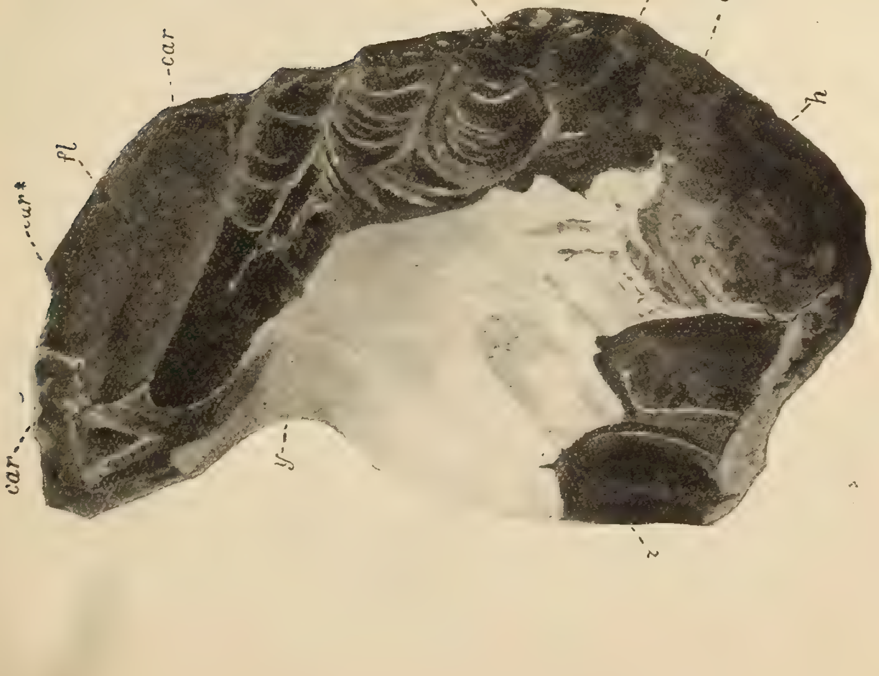

FIG. 13.

areas of the sculptured surface as the transposed and modified representative of the ventral plane and the dorsal plane of a fully developed rostro-carinate.

Dr. Peake informs me that the bed from which this and other similar flints come is mapped as "plateau-gravel" (Sonning Common, Oxon). It is made up of sand and gravel, about 10 feet thick, resting on the chalk. Here it is 250 feet above sea-level. Palæolithic implements were found by Dr. Peake on the surface in the neighbourhood, but none were obtained by him *in* this particular bed of gravel.

Section V.—*The Moir-Martlesham Jack-plane.*

In the Oise and Burnham specimens we have seen the rostro-carinate ideal type modified by the removal of the ventral plane to a lateral position, yet still recognisable. In the Sonning uncinate not only has the area, which seems to correspond to what was "the ventral plane," moved to an entirely lateral position, but the *dorsal* plane is not certainly recognisable. The rostrum—beak-like, uncinate, and dominant—is the leading feature of utility and permanence in those specimens.

I now have to draw attention to a specimen obtained by Mr. Moir from the sub-Crag detritus bed at Martlesham, near Woodbridge, Suffolk,* which is of considerable importance, *first* because of the unchallenged evidence of purposive and skilful human flaking which it presents; *second*, because of its undoubted *provenance* from the sub-Crag bed; and, *third*, because it presents only one of the *useful* features of the rostro-carinate type. It is *not* rostrate and it is *not* carinate, but it has a wonderful smooth, flat, ventral plane, 5½ inches long by 3¼ inches broad; the mass of flint surmounting this plane surface has been flaked into the shape of a pair of domes or prominences, the hinder of which gives an ideal grip for the right hand when the implement is used as a "jack-plane." One margin of the plane retains even to this day a fine cutting edge—and I therefore consider this as the anterior border—that which was moved forward when the implement was used—as I little doubt that it was—for shaving down wood. The whole "balance" of the heavy implement suits it perfectly for use in this way.

The two "domes" are separated by a natural valley clothed with original cortex. It is marked *cort.*[1] and *cort.*[2] in figs. 16 and 17. Another patch of cortex, *cort.*[3], is seen in fig. 16. A whitish calcareous incrustation covers the steep, almost vertical, surface of fracture (*vert.*, fig. 16) which rises from the

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* It was found by Mr. Moir's workman, Baxter, resting upon the London clay beneath shelly Red Crag in a pit in the nursery garden of Mr. R. C. Notcutt at Martlesham, in the year 1912.

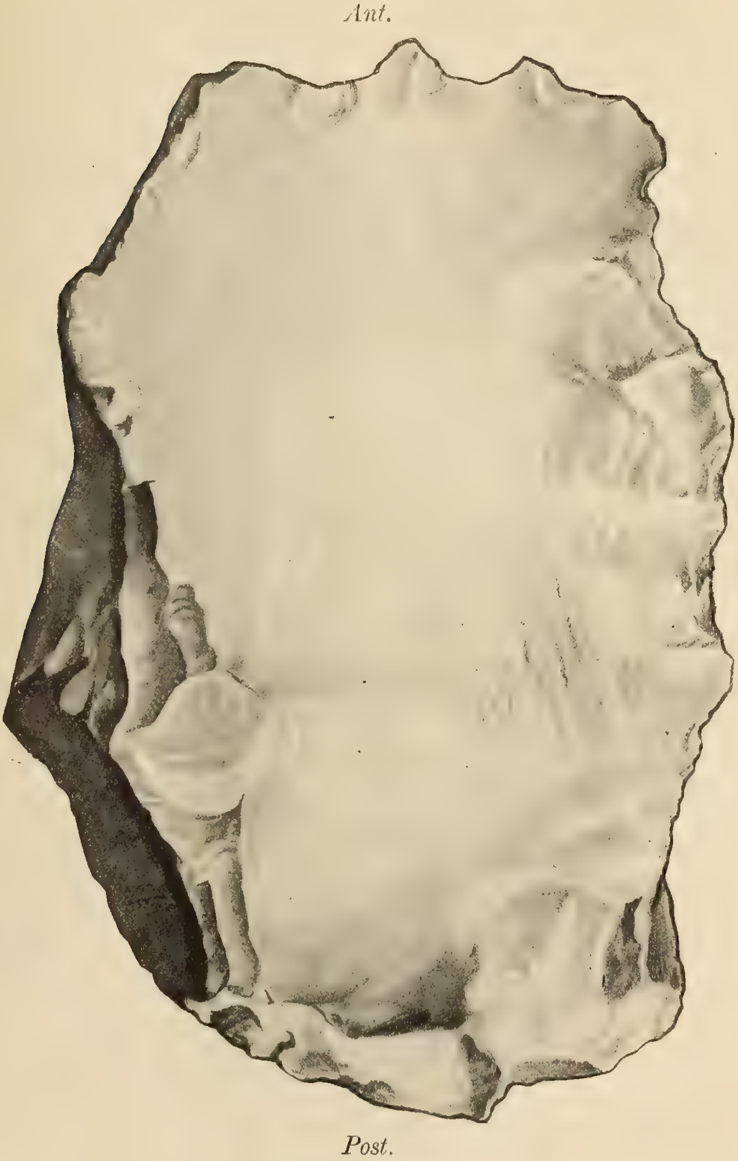

Fig. 15.—Ventral aspect of the "Martlesham Jack-plane." Drawn of the actual size. *Ant.*, anterior margin (apparently that which was used as a cutting edge when the implement was pushed forward in the manner of a carpenter's plane); *Post.*, posterior margin. The formation of this ventral plane by one large fracture, from the edges of which a few relatively small "trimming" flakes have been removed, is evident.

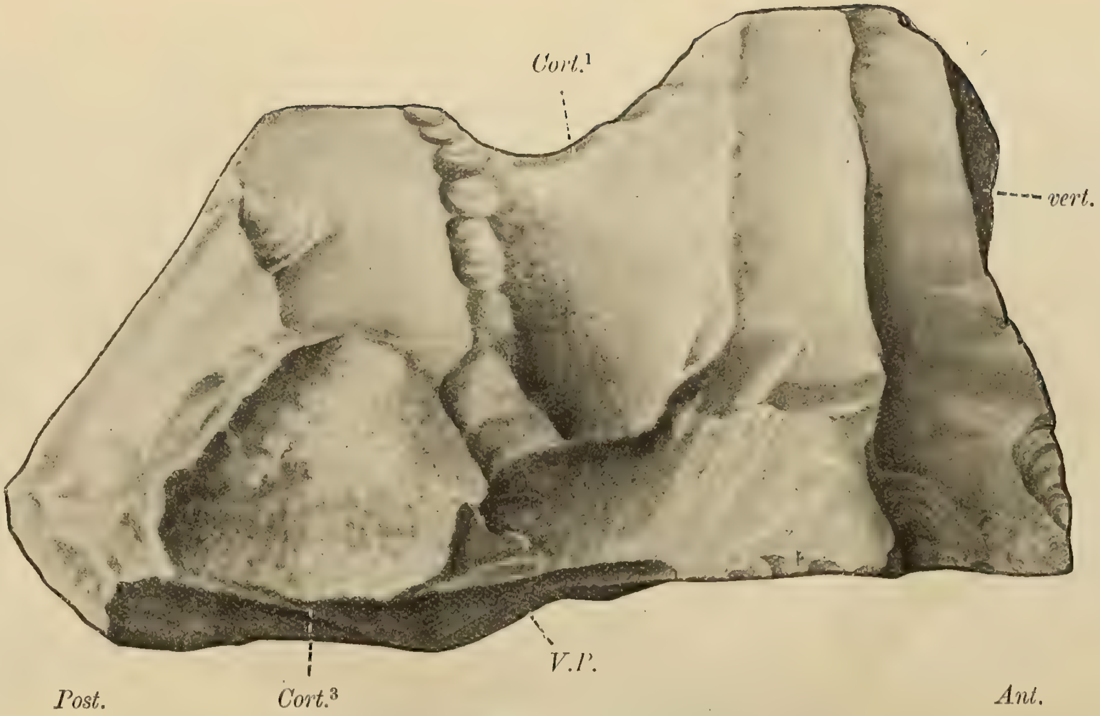

Fig. 16.—Right lateral face of the Martlesham Jack-plane.

Lettering in both figs. 16 and 17: *Ant.*, anterior; *Post.*, posterior; *Ant. H.*, anterior hump; *Post. H.*, posterior hump; *V.P.*, ventral plane (shown by slight tilting of the specimen); *cort.*^{1} and *cort.*^{2}, unflaked cortex in the valley separating the two humps; *cort.*^{3}, unflaked cortex on the right lateral area; *vert.*, vertical anterior face formed by a vertical fracture. The numerous large flakings by which the implement was sculptured and the ripple-marks showing their centres of percussion and direction are obvious.

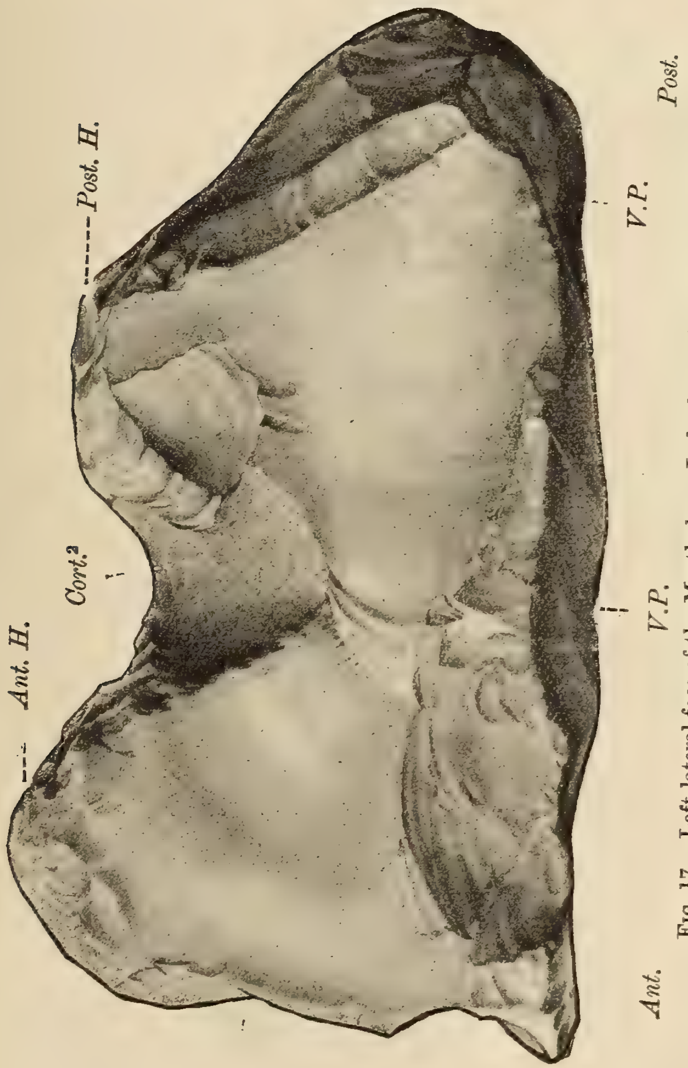

FIG. 17.—Left lateral face of the Martlesham Jack-plane. Letters as in fig. 16.

The cutting of the reference lines to *V.P.*, *Ant. H.*, and *Post. H.*, has unfortunately broken the outline of the drawing in figs. 16 and 17, as also have the reference lines to *car.* in figs. 10 and 11.

anterior cutting edge of the ventral plane. This fracture probably existed before this lump of flint had fallen into the hands of a human artificer. The rest of the surface presents a beautiful combination of milky greys and browns (due to iron-stain and decomposition*) and in some parts a brilliant polish (not rightly called "glaze"). Very numerous shallow and broad scratches cross one another on the flat surfaces of the flint. I am unable to decide whether they should be attributed to glacial agency or to less forcible sand scratching, which could, no doubt, cause markings on flint which had been exposed to the softening effect of atmospheric weathering.

Some day we may hope to see a careful and really adequate study of the scratching, polishing, staining, and other markings of flints from different deposits and of different antecedents, also a study of the fracture of flint by heat, by cold, by natural blows, by humanly directed blows—a very large subject, including hinge-fracture and an endless variety of "rippling"—but not admitting the possibility of the fracture of flint by the weight of superincumbent water or sand.

All the specimens figured in this paper, with the exception of Mr. Moir's "Whitton-Mid-Glacial typical form" (figs. 5*a*, 5*b*, 5*c*) and Dr. Peake's Sonning uncinate (figs. 13 and 14), are now presented to the British Museum Department of Ethnology and Mediæval Antiquities, where they can be examined alongside of the rostro-carinates previously described by me, and kindly presented by their owners to the national museum, chief among whom is Mr. Reid Moir, of Ipswich.

Theoretical Suggestion.

In conclusion I wish to point out that although it is not possible in the present state of our knowledge to gain direct evidence of the *historical* relationship and derivation, one from another, of the early forms of flint implements—there is yet sufficient ground for holding it to be probable that the conception of a "form" or "type" to be aimed at by the worker in flint, developed gradually, and that there *is* a real connection of the various forms which were manufactured. It is legitimate to assume as a hypothesis that the most generalised form—the rostro-carinate with a ventral "planing" surface—cutting latero-ventral edge, sharp-cutting carina or keel and pointed rostrum for grubbing and boring—in fact the form shown in fig. 1, is, if not the initial conception, yet at any rate that to which all others are connected.

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* The whitening of flint by solution of the opal which cements its microcrystalline siliceous particles needs some other name than "decomposition." I propose to call it "lactescence" and to describe the flint thus changed as "lacteate" or "milky."

PROCEEDINGS
OF
THE ROYAL SOCIETY.

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BIOLOGICAL SCIENCES.

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From it (fig. 1) we can imagine the flint-worker developing—as group 1—the more flattened "batiform" implement (of rare occurrence), still showing a median dorsal carina, and in contrast to this—as group 2—the platessiform (Chellean or Acheulian) implements with the carina forming one lateral margin and the ventral area *either* flaked down to form the opposite margin *or* translated into a lateral position. From the most generalised rostro-carinate (fig. 1) by curvature of the rostrum, often followed by dislocation or complete "atrophy" of the ventral and of the dorsal plane (as in the transitionals to the platessi-form type), we get group 3, the "uncinate" simplified type, figured in this paper. And lastly, as the exact complement of this we get group 4—a form showing the complete atrophy of rostrum and carina, with full and emphatic development of the parts atrophied in group 3. The type of group 4 is the heavy and useful "jack-plane" figured in the present paper.

Probably at any time the flint worker habitually made such flaked pieces and chipped forms as the piece of flint which he picked up and his own consciousness of skill suggested to him. At the same time there is evidence of such relationship in his mind and intention, in regard to the forms manufactured by him, as I have here sketched. There were probably "schools" and traditions in this art succeeding one another, and influenced by what had been established practice.

The passage from simple flakes to the side-scrapers and spear-head-like Moustierian implements is readily understood, whilst there are easy steps leading from Chellean heavy-butted hand-axes to the rougher Neolithic pieces and so to the fine chisel-like or adze-like "celts," both rough and polished, of Neolithic and modern stone-weaponed man.

On the Occurrence of Diatoms on the Skin of Whales.

By A. G. Bennett.

With an Appendix by E. W. Nelson.

(Communicated by Sir S. F. Harmer, K.B.E., F.R.S. Received June 10, 1920.)

In the course of my official duties during whaling operations at the South Shetlands and the South Orkneys, extending over four seasons between 1913 and 1919, I have observed that whales, and particularly the very fat individuals, are frequently covered with a film of a buffish colour. This film is easily removed, but owing to the excessively dirty conditions under which whaling is carried out, it is not an easy matter to obtain it free from admixture with other substances. The Blue Whale (*Balænoptera musculus*) and the Fin Whale (*B. physalus*) are species which are often thus coated. The film is equally thick on all parts of the skin, and it is easily seen on the white parts of the body. It is not nearly so obvious against the background of the darker parts of the skin, where it may easily be overlooked. A Blue Whale in this condition is known to the whalers as a "Sulphur-bottom."

It did not at first occur to me to investigate the cutaneous coating in question, but on examining microscopically, with a low power, a portion from a Fin Whale killed at the South Shetlands late in the season 1918–1919, I found it to consist of large numbers of what appeared to me to be Diatoms. When fresh, each of these organisms was seen to contain a yellowish speck of what seemed to be oil, near its centre. A small quantity of the film removed later from the skin of a Blue Whale, killed at the same locality, proved to agree closely, in its microscopical characters, with what had already been studied in the Fin Whale.

My attention has been called to certain observations recorded in the papers, relating to the whales of South Georgia, which were left by the late Major G. E. H. Barrett-Hamilton, and are preserved in the British Museum (Natural History). I am indebted to Mr. M. A. C. Hinton, who edited these papers in 1915, for the opportunity of quoting the following extracts from his completed Memorandum on the subject, which was printed though not actually published.

Under the heading of the Finback (or Fin Whale) Major Barrett-Hamilton stated that in four of the nine cases in which he had observed parasites, the parasites were "yellow algæ." Under the Blue Whale he recorded the following observations:—

On nine specimens the skin was covered with a 'yellow slime' believed to be *algæ;* No. 44, female, had the upper side of the pectoral sulphureous in hue, 'apparently from *algæ*, which also are frequent on underside'; they were also present on Nos. 42 and 43."

Although there is no evidence to show what further use Major Barrett-Hamilton would have made of these observations, if it had not been for his regretted death at South Georgia, it may be assumed that they refer to the same facts which I have myself noticed.

I have not at present had an opportunity of making a microscopic examination of similar skin-deposits in other species, but the following observations suggest that the occurrence of a superficial investment of Diatoms is by no means restricted to the Rorquals. During a whaling cruise in Orleans Channel, in about lat. 64° S., long. 61° W., the "Killer" (*Orcinus*) was excessively abundant, many of them coming alongside the ship. The light parts of all that were close enough to see clearly were of a distinct buff colour, sufficiently pronounced to be visible even while the animals were submerged, and closely resembling that of the corresponding parts of the Fin Whale and Blue Whale. A "Bottle-nose" (probably *Hypéroodon*), killed at the South Orkneys in 1915, had a similar colour, probably due to the same cause. I have not noticed this colouring in the "Humpback" (*Megaptera nodosa*). This might easily be overlooked, however, since the number of these animals which have been killed, in recent years, has not been large, and most of them have been almost entirely black.

The same colour is extremely common on most pieces of sea-ice, at water-level, in Antarctic waters, and is sufficiently pronounced on icebergs to form straight and wide bands, which remain *in situ* after the iceberg has tilted to another angle. I have not at present had an opportunity of making a microscopic examination of this material.

As Diatoms are abundant on ice, and the bands, presumably formed by them, have a similar colour to that of the Diatoms found on whales, I came to the conclusion that the colour was due to the same cause in both cases. The whales visibly infected are, almost without exception, very fat, a condition they are known to acquire when they have been feeding for some time on the food which is abundant in these icy waters, and from which, it appears to me, they probably receive their coating of Diatoms. Individuals of both the Blue Whale and the Fin Whale are abundant that look extremely clean, and these are usually poor in oil. I have long considered them to be new arrivals from warmer waters, but there is no evidence to show whether these individuals are coated with Diatoms from other seas.

I early saw that, should these Diatoms from whales prove to correspond with those on sea-ice, it might reasonably be anticipated that all or almost all whales would be found, on closer examination, to be hosts to Diatoms, and that the extensive collection of samples of their cutaneous investment might be of material assistance in tracing their migrations.

Appendix to the above Note, by E. W. Nelson.

I have received, through the British Museum (Natural History) a series of samples which were collected by Mr. A. G. Bennett as evidence of the conclusion he has come to with regard to the nature of the yellow colour of certain Antarctic Fin Whales and Blue Whales. These consisted of four mounted slides, three tubes containing the dried epidermal film, and a small tube containing portions of the film, from either a Blue Whale or a Fin Whale, which had been treated with nitric acid. In every case the sample contained specimens of a Diatom belonging to the genus *Cocconeis,* Ehrb., probably belonging to a new species which I describe below as *C. ceticola,* n.sp. With one exception, this species constituted the whole of the film, and of course occurred in immense numbers. The exception referred to was the tube containing the material which had been treated with nitric acid; and this consisted of *C. ceticola* and *Navicula viridis* (Nitzsch) in about equal proportions, with a sparse admixture of another species of *Navicula*. *N. viridis* is usually a fresh-water species, but it has been recorded as marine by Petit,[*] who describes it as "très commune sur les Algues marines." This is the only record I can find of this species, or of the closely related species *N. major, N. nobilis,* and *N. dactylus* being found, presumably living, in marine material, although they sometimes occur in marine soundings.[†] It may be noted, however, with regard to this sample, that from evidence which has been supplied by Mr. Bennett it is by no means unlikely that the material from the skin of the whale was contaminated with material from another source during its preparation.

It thus appears that, with one exception which is doubtful, the whole of the material collected by Mr. Bennett consists entirely of great numbers of a single species of Diatom, in a state of absolute purity. This fact goes far towards showing that the association of the Diatoms with the whale's skin is more than an accidental one. Several of the samples were of a distinct yellow colour; and that of the dry samples corresponded with No. 171 of Klincksieck et Valette's "Code des Couleurs." The samples were all from Blue Whales or Fin Whales collected at the South Shetlands in 1919; and those that were distinctly dated were obtained either in February or in March. These are dates on which the whales had probably spent a considerable time in Antarctic waters, in agreement with what is said by Mr. Bennett above.

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[*] Petit, P., "Diatomées récoltées dans le voisinage du Cap Horn," 'Miss. Sci. du Cap Horn,' vol. 5, Botanique, p. 121, Paris (1888).

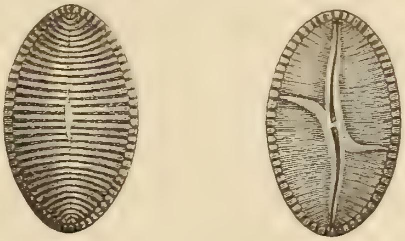
[†] See Mann, A., "Diatoms of the Albatross Voyages," 'Contr. U. S. Nat. Herbarium,' vol. 10, Part V, p. 359, Washington (1907).

The genus *Cocconeis* is characterised by having, generally, two different valves, the upper one being strongly convex and without a true raphe, and the lower valve being slightly concave and always bearing a true raphe. The plant lives attached to Algæ, etc., with the lower valve in contact with its host. It is capable of moving about to a limited extent. It is not a plankton-form, and when taken in the tow-net its inclusion is probably fortuitous.

Certain Diatoms are known to take on a saprophytic mode of nutrition under suitable circumstances. This is usually accompanied by a more or less profound change in the colour of their cell-contents. The colour can only be seen in the fresh, living material, and it may be hoped that Mr. Bennett will have the opportunity, on his return to the Falkland Islands, of recording it more accurately. It would be desirable to compare the colour of the Whale-Diatoms with that of other Diatoms living under other conditions in the same region.

Cocconeis ceticola Nels., n. sp.

Valvis late lanceolatis ellipticisve, 18–30 × 12–18 μ; valva superiori valde arcuata, striis subradiantibus ordinatis ornata, 10–15 in 10 μ, striis subtilissime punctatis, quarum puncta haud vel ægerrime conspicienda; pseudorhaphe angusta, recta, in medio leviter dilatata: valva inferiori coronula intra marginali costarum valde abbreviatarum instructa, hinc inde striis debilissimis ad centrum evanescentibus ornata; rhaphe leviter subsigmoidea, utroque apice breviter transverse ramosa; area centrali oblique dilatata, in diversas partes axis transversalis ad marginem recurvata.



1. Upper valve. 2. Lower valve.

Cocconeis ceticola n. sp.
× 1000.

Exemplum No. 33771, *in Musæo Britannico, Londinii.*—Hab. in cuti cetorum, *Balænoptera musculus* et *B. physalus*. South Shetlands (found in all Mr. Bennett's samples).

The striæ on the upper valve, under the best conditions and with oblique light, could not be resolved into dots, but had a strong appearance of being punctate. The material examined did not permit any description of the cell-contents. Minute examination of the figure will show certain markings which are inherent to the process of reproduction, and are not intended to represent structure seen in the specimens. As explained above, no dotted structure could be made out, and the lower valve within the border is here shown far too strongly striate.

I desire to thank Mr. E. Ford for kindly making the drawings which illustrate this note.

Navicula viridis (Nitzsch) Ehrb.

Ehrenberg, 1838. 'Infus.,' p. 182, Plate 13, fig. 16 (in part), Plate 21, fig. 12.

Kützing, 1844. 'Bacill.,' p. 97, Plate 4, fig. 18; Plate 30, fig. 12.

Petit, 1888. 'Mission Scientif. du Cap Horn,' vol. 5, p. 121.

Mann, 1907. 'Diat. of the Albatross,' p. 359.

Bacillaria viridis Nitzsch, 1817. 'Neue Schrift. Naturf. Ges. Halle,' vol. 3, p. 97, Plate 4, fig. 1–2.

Found only in the tube containing the material which had been treated with nitric acid.

It is quite impossible to define the exact limits between this species and *Navicula major* Kütz, and these specimens might be referred to the latter with almost equal reason.

The name has, however, been chosen as the oldest specific designation which adequately describes the organism, and also since this species has already been reported from Cape Horn by Petit (*loc. cit.*).

This species is normally found in fresh water and the above is the only known record where this Diatom has been found *living* in salt water. It has been recorded from marine soundings (see Mann, *loc. cit.*) but this fact does not necessarily prove a marine habitat when it was a living organism.

As explained above, it is not quite certain that this and the following species were really derived from the skin of a whale.

Navicula sp.

Pinnularia interrupta W. Sm.

Smith, W., 'Brit. Diat.' 1853, p. 59, Plate 19, fig. 184; 1856, p. 96.

Cleve, P. T., 1895, 'Kongl. Sv. Vet. Akad. Handl.,' 27^3, p. 76.

Pinnularia biceps Greg.

Gregory, W., 1856, ' Q. J. M. S.' IV, p. 8, Plate 1, fig. 28.

This form was sparsely represented in the sample containing *Navicula viridis,* and in no others. These specimens are smaller (30 μ) and less robust than the type, and the costæ are not completely interrupted. If, as here, the genus *Pinnularia* is not admitted, the specific name *interrupta* is not available, being pre-occupied by Kützing (1844). The taxonomy is further complicated by the use of the specific name *biceps* by Ehrenberg (1843), for a form differing from Gregory's. A complete discussion of the synonymy would be outside the scope of this paper.

It is desirable to investigate the question of the occurrence of Diatoms on the skin of whales further, and suitable material would be gratefully received by the British Museum (Natural History). For this purpose numerous samples should be collected, from various localities, or from the same locality at different seasons of the year. A careful record should be kept of the species of whale, with the locality and date. Special care should be taken to obtain samples which have not been contaminated by contact of the whale's skin with foreign bodies. Material of similar appearance should be collected, if possible, from ice, algæ, wood, shells, stones, or other submerged objects. The method of preservation recommended is as follows: To every 250 c.c. of the material in sea-water add 10 c.c. of strong Flemming's solution (15 volumes of 1 per cent. chromic acid, 4 volumes of 2 per cent. osmic acid, and 1 volume of glacial acetic acid), and store in stoppered bottles. A simpler procedure is to put the material direct into 10 per cent. formalin solution.

Croonian Lecture:—*Genetic Segregation.*

By W. Bateson, M.A., F.R.S.

(Received September 20,—Lecture delivered June 17, 1920.)

The later developments of Mendelian analysis have been in the main an attempt to elucidate the scope and nature of Segregation. Mendel proved the existence of characters determined by integral or unit factors. Their integrity is maintained by segregation, the capacity, namely, to separate unimpaired after combination with their opposites. Our first aim has been to discover specifically what characters behave in this way, whether there is any limit to the scope of segregation, or any characters or classes of characters which are determined by elements unable to segregate simply. The second object has been to decide the time and place in the various life-cycles at which segregation occurs. It is with the latter problem that I propose to deal more particularly in this Lecture, but a brief consideration of the range of characters, subject to segregation, is appropriate.

1. *The Scope of Segregation.*

Of the classes of features by which animals and plants are distinguished, most have now been shown to be dependent on segregable elements. It is perhaps noticeable that we have no quite clear proof that the factors governing differences in number, meristic characters in the strict sense, commonly behave so simply as those determining other characteristics. There are examples of parts repeated in series, such as the extra toe of the fowl (a dominant) and the leaf of the monophyllous Strawberry with a single leaflet (a recessive) which have a factorial inheritance, but the resulting terms, especially the heterozygotes, are indefinite. In the polydactylous fowl, for example, the heterozygote has a great variety of shapes. The hind toe is most often represented by two sub-equal digits, but the duplicity may be so slight as to appear externally only as a division of the nail. It may also assume another very different form, in which there is only a single many-jointed digit representing the usual pair. In the monophyllous Strawberry the homozygous recessive, whether before or after immediate extraction from the heterozygote, shows fluctuations to a 2- or 3-leaved condition.* Such fluctuations are common among forms distinguished by meristic peculiarities. There is not the uniformity and simplicity which is such a striking feature of variations in colour and many other substantive characteristics. The evidence as to meristic differences is however still scanty, and it is too soon to decide what importance should be attached to this preliminary impression.

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* Richardson, C. W., 'Jour. Gen.,' vol. 3, p. 171 (1914).

With more confidence we recognise that merely quantitative differences seldom, if ever, have a perfectly simple inheritance. There are two obvious interpretations: (1) that the factors do not usually segregrate clean; (2) that the number of factors involved is so large that their effects are masked. Adequate discussion of these two possibilities could only be given at considerable length. On the whole, I incline to the former alternative, but the material for a decision scarcely exists as yet. Certain examples should be noticed in which, though the most obvious differentiating feature is quantitative, the underlying physiological distinction is more probably to be referred fo a qualitative factor. Height in certain plants is a good illustration. It is ostensibly a quantitative feature, and the talls segregate clean from the dwarfs. But in various cases, *e.g.*, Peas and Sweet Peas, the dwarfs are also a darker green. The dwarf of *Campanula persicifolia*, especially (about 8 inches high), is a plant so strikingly different from the type (2–3 feet high), that it is sold as a species "*C. nitida.*" The leaves of the dwarf are an intense, dark green.* This variety is, of course, a recessive and segregates without intermediates, yet, if the qualitative distinctions were less evident it might easily be classed as a variety in quantity. But the critical distinction is certainly qualitative and the great difference in size is consequential. Though in such cases segregation is complete, it may almost be said to be characteristic of purely quantitative distinctions that one or other of the original parental types fails to reappear in its extreme form after a cross. So usual is this feature in quantitative segregation that the phenomenon must have special significance.

Another fact is beginning to emerge which must contribute to the shaping of a conception of the physiological nature of segregation. We have learnt that groups or complexes of factors may segregate whole. To such a complex the distinction in sex is due, but in certain cases it may break up. The occurrence of a large spur in fertile hens (Leghorns, for instance) must be regarded as due to the absence of that part of the sex-complex which in the normal hen inhibits the growth of the spur. In ordinary fowls the whole inhibiting group remains on the female side, but the spur-inhibiting element can evidently separate from the rest. On the other hand, when in the cocks of certain breeds (as occasionally in Wyandottes) there is no spur, we

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The ovary projects in a curious way above the sepals so much, that were the plant not a *Campanula*, botanists would describe this ovary as superior.

may perhaps conjecture that this element has been transferred to the male side.*

The presence of the characteristically masculine comb and wattles in the male Sebright, which is otherwise henny, shows that another factor similarly detachable governs their development.

To the breaking up of large compound factors the production of intermediate types, such as occur among the colour-varieties of plants, is in all likelihood due. The Sweet Pea and the Snapdragon have now an innumerable series of such colour-forms which may be represented as having arisen by the disintegration of the various anthocyanins. That, at least, is the simplest way in which their origin can be conceived.

To the final result many qualifying elements contribute, and these may naturally be separate entities. But change in the amount of the same colouring material, and diminution in the mere extent of its distribution, are common features of these graduated series. As the cultural development of the species progresses, more and more of these quantitative intermediates appear, and are selected, until a practically continuous series is produced. Although the interrelations of the whole series can be represented by a factorial scheme, the assumption that each of these factors had an aboriginal individuality appears gratuitous. In *Antirrhinum*, for instance, the ordinary self-coloured flower segregates as a single unit from the white. But there are "Delila" forms having the "face" coloured and the "throat" white. Another variety has the "lip" coloured and the peripheral parts white, and to this again there is an almost exact counterpart in which the peripheral areas are coloured and the lip nearly white, and between these again there are further intergrades. Apart from factors modifying its quality, the colour of the whole corolla, segregating as a single entity from the white, would without hesitation be represented as depending on a single factor. Subsequent experience that this entity can break up into an indefinite nnmber of fractions is not evidence that the original representation was wrong. This reasoning applies to a great range of phenomena.

In view of the chromosome theory of linkage, it is therefore worth remarking that we do not find linkage especially frequent among these fractional factors. Have they, then, been distributed among different chromosomes? If in *Antirrhinum*, the colour of the face and of the throat were lately parts of a single factor for the total flower-colour, would not linkage between them be expected? Nevertheless, in cases of this sort, so far as I know, linkages have not been found with any special frequency.

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* After much experiment the genetics of spur-development are still very obscure. In the course of a long series begun by crossing spurred Leghorn hens with a spurless Wyandotte cock (giving F_1 normal in both sexes), neither form has reappeared in F_2 with its original development. Very rarely a hen with minute spurs has appeared, and occasionally a cock with the spurs sensibly reduced. Nor from spurred hens $\times F_1 ♂$ has anything approaching the original types been raised. Conceivably the detached element is able to join again with the rest of the female complex.

The segregation of a group of differences—and presumably factors—in combination has lately been shown by Renner* to occur with extraordinary frequency in the Œnotheras, and this peculiarity is without doubt at the bottom of the difficulties which have beset the genetic analysis of these plants. The complexes are in several forms or species not borne equally by the two sexes of the same plant, and most of them are unable to exist in the homozygous state. These discoveries greatly elucidate the Œnothera problem. For instance, not only *Œn. lamarckiana*, but *biennis*, *muricata*, and others also, are not homozygous types, but heterozygotes of a special kind. Consequently, the production by them of "mutants" is not capable of the simple interpretation originally applied to them by de Vries. Renner suggests that the mutants arise owing to some interchange between the complexes which at present we cannot investigate, but whatever be the exact manner of their origin we cannot regard them as genuine examples of the production of novel forms by a homozygous type.

Before leaving this part of the subject, we may notice that the supposition that segregation is concerned solely with characters of a superficial or trivial nature has been long ago disproved. Baur's Antirrhinums, the study of which was continued by Lotsy, were an excellent demonstration to the contrary, for they provided many illustrations of segregation in features, the "specific value" of which no systematist would question. If further evidence were needed, it may be found in the fine series of experiments lately published by Heribert-Nilsson† in *Salix*, which, contrary to the belief long ago entertained by Wichura, show that, when F_2 families are raised in adequate numbers, very striking segregation occurs in the species-crosses. Many geneticists are inclined to the view that segregable characters should be pictured as implanted on an irreducible base which is outside the scope of segregation, but no means have yet been devised for testing the reality of this conception.

2. *The Moment of Segregation.*

The next question is to determine when in the various life-cycles segregation can occur. Obviously it is a phenomenon of cell-division. If we knew nothing of the genetics of plants we might confidently adopt the view which Morgan has so successfully developed, that normal segregation and redistribution happen exclusively in the process of reduction. Though unconvinced, I cannot deny that linkage and crossing-over may well be represented provisionally, as effected during synapsis. The scheme previously offered by Punnett and myself as a diagrammatic plan capable of representing these phenomena is certainly far less attractive. There is evidence that in certain plants, *e.g.*, *Matthiola*, the composition of the families derived from single pods shows very great and perhaps irregular fluctuations, and the normal ratios for those families is only found by taking the average of many, but no sufficient statistical examination of such cases has yet been made. In some suitable case estimations of the offspring derived from individual anthers might be of value in this connexion. Renner, by examining the starches of the pollen-grains in Œnotheras, has lately made visible that di-morphism, of which we had previously genetic proof, and perhaps this novel and striking observation might be used for the purpose of mapping the distribution of such a character among the pollen-grains. Meanwhile, it must be granted that no indication that gametic linkage results from somatic differentiation has yet been obtained.

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* 'Zts. f. ind. Abstammungs- und Vererbungslehre,' xviii, 1917, p. 121.

† Lund's 'Universitets Årsskrift,' N.F., Avd. 2, vol. 14, November 28, 1918.

When, however, we examine the view that linkage of factors is a consequence of their association in a chromosome, we must observe that there is no body of evidence that the number of linkage-systems agrees with that of the chromosomes, a primary postulate of Morgan's theory. *Drosophila* is the only example which has been adequately investigated. The cytological appearances are not readily consistent with the other postulate of Morgan's case, that crossing-over is effected by anastomosis of chromosomes and exchange of materials between them. In our present ignorance of the physical nature of the factors we are not obliged to assume that an actual transference of material is an essential condition for an exchange of properties; but since Morgan's suggestion is made in that form we are bound to notice how difficult it is to interpret the visible phenomena of cytology in accordance with that hypothesis. Without personal familiarity with cytology no one can have a confident opinion. I observe, however, that in his most recent publication on these subjects, E. B. Wilson* gives a very emphatic "counsel of caution," remarking that writers on genetics have taken too much for granted, and that for the present the genetic development of the chromosome theory has far outrun the cytological. To a layman the visible appearance of chromosomes is scarcely suggestive of the prodigious material heterogeneity demanded, and the general course of cytological evidence seems to indicate that the *rôle* of the chromosomes is passive rather than active. Though showing features of regularity, they are capable of very wide variations without transgressing the limits of viability, which could scarcely be the case were every detail in their organisation critical. The appearance of chromosomes is not to me suggestive of strings of beads of extreme heterogeneity, but rather of strands of some more or less homogeneous substance; and in so far as numerical and geometrical order is exhibited by them, it would, in my opinion, be more proper to compare this regularity with that seen, for example, in drying mud or in the formation of prisms of basalt than to attribute to it a more fundamental meaning.

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* 'Amer. Nat.,' p. 210, May–June, 1920.

Leaving these speculative considerations, and limiting our inquiry to the concrete question, at what moment in the cycle does genetic segregation occur, we reach a perfectly definite answer: that whatever future research may decide as to the occurrence of segregation in animals—which, for aught we know, may always be effected at the reduction division—there is no such limitation in plants. We are now thoroughly familiar with a large group of examples in which the genetic properties of the male and female cells of the same plant are quite different. In these, at all events, the reduction-division cannot be the moment of the segregation by which these characters are distributed.

The first case detected was in *Matthiola*, where Miss Saunders' results proved that in the double-throwing singles the pollen carries exclusively doubleness, the eggs being mixed, some single and some double. A similar condition was shown to exist in regard to the cream and white plastids respectively, the pollen grains bearing exclusively cream. De Vries observed a comparable arrangement among the Œnotheras, and Renner has lately shown that the phenomenon is widely spread in that group, thereby providing a consistent interpretation of much that was formerly obscure in the genetic behaviour of these plants. In *Campanula carpatica* Miss Pellew proved that the pollen grains of the hermaphrodite form called *pelviformis* carry exclusively femaleness, and preponderantly white flower-colour (the plant being heterozygous for blue). The case of Petunia investigated by Miss Saunders* is somewhat peculiar in the fact that in the heterozygous singles the male side carries the dominant singleness only, since in those instances to which the conception of dominance can be applied, it is the male which commonly carries the recessive. Segregation of these characters cannot in plants so organised be supposed to take place later than the constitution of the male and female organs, and therefore the reduction division cannot be the one critical moment. The suggestion has been made that germ-cells of the missing kinds may be in reality formed and perish before reaching a functional stage. As regards the Œnotheras, where shrivelled pollen grains abound, this conjecture is very plausible and probably correct; but when as in the other cases here quoted, the pollen grains are uniformly sound, the hypothesis is inapplicable and without evidential support. Moreover, even if it were true that certain classes of germ-cells perish in one or other of the sexes, that would hardly alleviate the difficulty, for this differential viability would remain to be be accounted for, being itself a phenomenon of segregation.

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* 'Jour. Gen.,' vol. 1, p. 57 (1910).

*Begonia Davisii** is another curious illustration in which segregation must occur even earlier. This plant is a wild, true-breeding species, with ordinary single flowers. All the pollen grains however carry doubleness, and used on the female flowers of doubles give offspring all double (single being the dominant). The pollen of this plant is as uniform and perfect as those of any species I have ever seen. We must therefore conclude that the segregation by which singleness separates from doubleness is effected not later than the formation of the rudiments of the male and female flowers. Cytological investigation may no doubt show that the distinctions between the genetic properties of the male and female are associated with visible nuclear differences, but I see no reason for anticipating that such differences must exist. Cells which differ in their genetic potentialities must of course differ in physical constitution, but there is no reason to suppose that this difference need be in any way dependent on chromosome structure.

As regards *Campanula carpatica* "*pelviformis*" and *Begonia Davisii* experiment has shown that the peculiar kind of segregation which they exhibit may recur in their offspring. In the *Begonia*, if the female of *Davisii* be fertilised with pollen of an ordinary double tuberous *Begonia*, the doubleness so introduced stays on the male side just as the doubleness of its own male does, and a plant so bred has its pollen all double. But if the male of *Davisii* be used on the female of an ordinary single, there is no restriction of doubleness to either sex of the offspring. The peculiarity of *Davisii* must therefore be attributed to the special properties of its female side. The *Campanula* case is complex and has not yet been fully explored, but at least from the female side of *pelviformis* plants have been raised which retain the properties of the mother as regards the distribution of the white and blue colours.

We have at the John Innes Institution been lately investigating a similar case in flax, which though comparable has some special features. A dwarf flax (*Linum usitatissimum*) of unknown origin, presumably a stray seedling of one of the varieties grown for oil, was fertilised with pollen from our tall fibre strain. Both parents breed true to the fully hermaphrodite condition with anthers perfectly formed, and the F_1 plants were normal in this respect. F_2 consisted of hermaphrodites, and a recessive form with aborted anthers, generally contabescent and not dehiscing at all, but having occasionally a few grains of good pollen. The ratio was a normal 3 : 1. The recessive, having occasional grains of pollen, self-fertilised, gave similar plants with anthers wholly or almost wholly aborted. The normal F_2 hermaphrodites gave in F_3 families which showed that some of the F_2 plants were pure normals, others heterozygous in the ordinary way. But when the recessives were fertilised with pollen from three several varieties of tall fibre flax, only recessives were produced. These tall flaxes therefore are normally heterozygous for the recessive or "sub-female" condition, and this in segregation is permanently relegated to the male side of the plant, while the female side takes the hermaphrodite factor. Segregation in regard to the same recessive may take place in one of two ways. It may be *unilateral*, as it is when in heterozygous association with the female of the tall flaxes, or it may be *ambilateral* and unrestricted to either sex when it is in association with the female of the oil flax. We must infer that the female halves of the two types differ in some critical respect which decides the manner of the segregation.

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* 'Jour. Gen.,' vol. 8, p. 199 (1919).

Unilaterality may also show itself as a difference in the closeness of linkage on the two sex-sides of the same plant, and no doubt this fact may have a bearing on the interpretation of the foregoing cases. The late R. P. Gregory discovered the first case of this, in *Primula sinensis*. He found that the linkage between magenta colour and short style was closer in the eggs than in the pollen. Recent work on a larger scale has given 10·9 : 1 as the female linkage and 6·4 : 1 for the pollen. A similar difference has been also found for the linkage between green stigma and "reddish" stem (as opposed to dark red), the value being 29·8 : 1 for the eggs and 41·7 : 1 for the pollen. In both examples, individual families show wide fluctuations, and these values should for the present be regarded as approximate only. Whatever be their meaning, they show that some segregation has occurred in the formation of the two sets of sexual organs, such that the process of gametic differentiation is not the same in both.

Besides these examples of differentiation between the male and female sides, there are others proving that segregation may occur at other stages in somatic development. The most obvious examples are the variegated plants. I have discussed this subject elsewhere in connexion with reversible periclinal "chimæras" of white over green which produce shoots having the white enclosed in the green.* To these must now be added the cases in which the plants arising from adventitious buds differ from the plants which produce them. I have described one of these examples in *Bouvardia.* The pinkish white "Bridesmaid" gives the red flowered "Hogarth" from its root-cuttings. Three similar occurrences have been found in fancy Pelargoniums. The root-cuttings of a white flowered variety, "Pearl," give a red-flowered form, very like "Mme. Thibaut." "Mrs. Gordon," which is a full rose-pink, with whitish edges, gives from its root-cuttings flowers in which the two posterior petals are marked with dark red, not unlike the variety "Cardiff." A more striking case is that of "Escot," which gives from its roots plants with bright pinkish red marks, those of the original being purplish red. The most curious feature of this case lies in the increased size both of the plant and the flowers coming from the roots, and it is scarcely possible to see the petals of "Escot," which are characteristically rolled back, side by side with those of the root-form, which are not only larger but also flat, without surmising that this rolling back is an expression of the greater size of the larger petal contained within the smaller, causing a want of correlation between the growth of the inner and outer tissues.

Buckling or crumpling of leaves through want of correlation was a conspicuous feature of some of Winkler's "graft-hybrids," made from *Solanum nigrum* and *S. lycopersicum,* when the larger tomato was enclosed within the smaller species. We have had a precisely similar example in a salmon-fringed Pelargonium bred by Mr. Jarman, of Chard. The leaves are obviously buckled, the petals are laciniated, and the female parts aborted, though the anthers are perfect. This male and deformed flower is proper to the outer tissues only; for on two occasions the plants have produced shoots with large flat leaves and normal hermaphrodite flowers with their petals entire. Obviously, this normal plant was enclosed within a skin of the fringed type.

In all these examples, a somatic segregation has occurred which determines the genetic potentialities. The interpretation that they are *periclinal* chimæras is probably correct for the most part. The fringed Pelargonium is obviously of this nature. Nevertheless, the fact that a root-cutting consistently produces a certain type of plant which is not the original does not prove that the distribution is periclinal. Another possibility is well illustrated by the case of a variegated *Spiræa ulmifolia,* having the stem, petioles, and (basal) centres of the leaves without chlorophyll.* The growing point has the power of laying down green tissue in the lateral areas only, the internodal regions being albinotic. Root-cuttings from this form give albino plants which die after the development of two or three small leaves. Now in this case we can see the distribution of the green and white respectively, and we recognise that the roots give albino plants because they belong wholly to the albinotic area. On similar lines it is possible to interpret the *Bouvardia* and other cases. The distribution of the two types in the same plant may be such that one is limited to the root and internodes, while the other is in the nodal structures.

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* 'Jour. Gen.,' vol. 7, p. 93 (1919).

That considerations of this kind are not fantastical is proved by the genetical phenomena seen in the case of "rogues" in culinary peas, which Miss Pellew and I have been investigating for a number of years.† The rogue is a peculiar, wild-looking plant, differing in various ways from the type, chiefly in having pointed leaflets. Crosses between it and the type give plants which in their lower parts are intermediate, though turning into rogues as they develop. The self-fertilised offspring of rogues and also of these F_1 plants are always rogues, and evidently the type-characters introduced from the type parent are left behind in the lower parts. Such a case may perhaps be compared with the condition seen in the variegated *Spiræa*, and we may fairly conjecture that if it were possible to raise root-cuttings from the F_1 peas, they would produce types.

A more gradual exclusion of the type-elements in the lower parts is seen in certain intermediates. These may scarcely differ from types in the lower parts, though changing to rogues, sometimes abruptly, sometimes gradually, as the series of flowering nodes is developed. Reciprocal crosses between the successive flowers of such plants and the flowers of types has shown that, together with the gradational change in the somatic structures, there is also a gradational change in the proportion of gametes bearing the rogue and type characters respectively. This proportion and the rapidity of the change differ on the male and female sides. Of the *egg cells* in the lower flowers, up to about the 10th flowering node, rather more than 50 per cent. carry the type-characters—or at least the non-pointed leaflets—but above this level the proportion of types declines. Of the *pollen* in the lowest two flowers only about 20 per cent. is type-bearing and the proportion diminishes rapidly in each successive flower above that level.

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* This is somewhat like the Pelargonium named by Messrs. Cannell "Freak of Nature," in which the chlorophyll has a closely similar distribution, and it, like the *Spiræa*, is sterile on both male and female sides. In this *Spiræa* I have never seen pollen, but very rarely a fruit is formed, which, no doubt, is due to an occasional development of a bud in the green area, an occurrence frequent in variegated plants. Whether these fruits contain viable seeds is not yet known.

† 'Roy. Soc. Proc.,' B, vol. 91, p. 186 (1920).

In all the examples given hitherto the segregation is in diploid tissues, but a comparable phenomenon has been proved by Collins to occur in the *haploid* axis of a moss (*Funaria*). In a diœcious moss, as the Marchals have shown, sex-segregation occurs at spore-formation, the division in which reduction is effected. This, of course, agrees with cytological expectation, though so far as I know, the details have not been observed. But from the leaves of mosses placed in nutrient fluids new plants may be raised without great difficulty, and Collins found that the (perigonial) leaves surrounding the male organ thus propagated, produce *exclusively male* axes.* He has since raised similar cultures from the (perichætial) leaves surrounding the female organ, and, as related in the paper following this, from them monœcious plants resulted. The proof is thus complete that in a haploid tissue a segregation of sex can occur.

The inference may be drawn that the factors for other characters may similarly be liable to segregate in the haploid state. In this connexion I may mention a case which though as yet obscure, perhaps fulfils this expectation. In botanic gardens a variegated maiden-hair fern (*Adiantum capillus-Veneris*) is grown which has wedges of white tissue irregularly distributed in the segments. This plant produces spores freely,† and these give rise to prothallia which in several cultures raised here have always been entirely green. But when ferns arise from these green prothallia by the sexual process, they are of three kinds, green, white or variegated like the parent plants. The fact that the prothallia should be all green is entirely unexpected and creates a distinct problem, but it is evident that segregation must occur either in some of the cell-divisions by which the prothallia proliferate, or in those by which the gametes are formed, in either case in haploid tissue. This segregation is essentially different from that by which the differentiation of organs, such as the archegonia and antheridia is accomplished, inasmuch as it relates to elements determining the characters of the next generation.

From the evidence given it is clear that in a wide view of living things segregation cannot be exclusively a property of the reduction-division, and for the present, it should be regarded as a possibility which may occur at any division in the life-cycle.

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* 'Jour. Gen.,' vol. 8, p. 145 (1918–9).

† I have not satisfied myself that spores are produced in sori on the white areas.

The Genetics of Sex in Funaria hygrometrica.

By E. J. Collins, M.A. (Cantab.), B.Sc. (Lond.).

(Communicated by W. Bateson, Esq., F.R.S. Received September 12, 1920.)

For diœcious mosses El. and Em. Marchal* have shown that sex segregation occurs at the meiotic division of sporogenesis, inasmuch as the individual spores which initiate the haploid gametophytic phase are uni-sexual, producing protonemata from which are developed leafy axes all of one sex either male or female.

In a former paper† the writer has shown that vegetative cultures derived from the antheridia and the surrounding "perigonial" leaves of the male "inflorescence" of *Funaria hygrometrica*—a monœcious type of moss—produced male plants only, whilst cultures derived from spores reproduced the normal monœcious plants.

From this evidence it appeared probable that at some point in the cell divisions by which the axis and its organs are developed, a separation takes place such that the element upon which the monœcious condition depends is dropped out of those cells from which the male organ with its surrounding leaves is formed.

It was therefore highly desirable that vegetative cultures should be made from the female sexual organ and the leaves surrounding it, their genetic potentialities being unknown. These experiments have now been carried out, vegetative cultures having been raised both from the archegonium and from the surrounding "perichætial" leaves of *Funaria hygrometrica.*

After the fertilisation of the egg, the flask-shaped archegonium enlarges considerably, the basal part (or venter) particularly becoming much inflated. In this condition it is quite easy to remove the greater part of the archegonium (the venter with the surmounting neck) for the purpose of experiment.

Cultures were made from the inflated venter and neck shortly after its appearance above the surrounding perichætial leaves and also from these leaves.

Four pot cultures in all were established on sterilised soil, two from the venter and two from perichætial leaves. All were placed under a cloche upon moist sand in a zinc tray, together with a fifth pot of soil sterilised in the same way, but upon which no culture had been sown. The pots were watered with boiled distilled water through an opening in the top of the cloche. No growth appeared in the control pot.

These cultures have produced typical monœcious plants.

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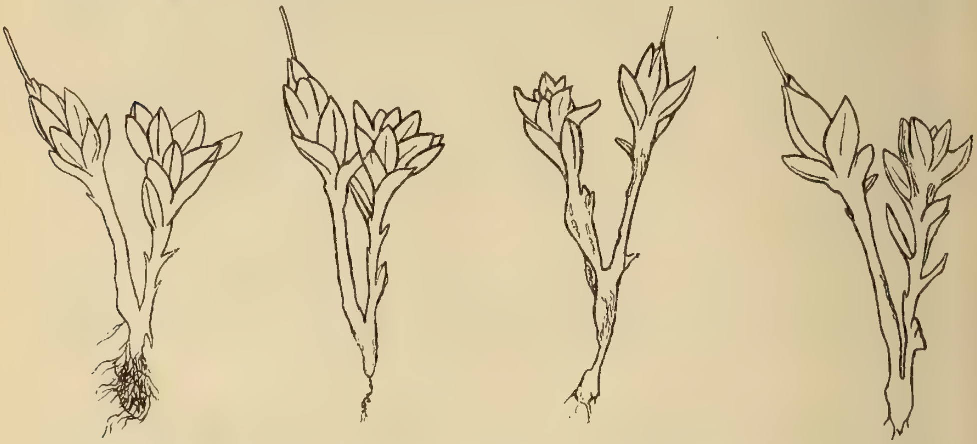
* 'Mém. de l'Acad. Roy. de Belgique,' 1906; 'Bull. de l'Acad. Roy. de Belgique Classe des Sciences,' 1907, 1909, 1911.

† 'Journal of Genetics,' vol. 8, 1919.

It appears evident, therefore, that up to the point of the formation of the female organ, the cells of the haploid gametophytic phase retain the power to produce monœcious plants, whereas the leaves surrounding the male organ have lost this power.

In *Funaria* in the early condition the shoot bearing the male inflorescence overtops the axis bearing the archegonia, and this appearance leads to the assumption that the female shoot is a lateral development. From specimens taken from my cultures (see text-figure) the point of origin of the female shoot appears variable, and in some cases the structure simulates a dichotomy. More rapid growth of the male axis for the very obvious purpose of facilitating fertilisation would lead to the axis bearing the archegonia assuming a lateral position. In view of the new observations as to the difference in potentialities of the male and female shoots, it becomes important to determine the morphological relation of the two parts and their exact cell-lineage. For at some critical cell division a change takes place: either the power to produce the monœcious plants is extruded in the female shoot, the deficient male axis continuing the development; or a bud which has lost this power is extruded as the male shoot, while the female shoot retains it and continues the development of the axis.



Tracings from photographs of four *Funaria* plants, showing relation of female shoot to male. In each case the female is distinguished by the protruding "neck."

In this connection it is interesting to observe that Miss E. R. Saunders has shown me some of her preparations of the termination of the female axis, which show the presence of antheridia among the archegonia.

The cytological aspect of the phenomena has not been studied, and the generally accepted alternation of generation within the group with its n and $2n$ phases has been assumed throughout.

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OF

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Photo by Elliott & Fry, Ltd.

F. D. Godman

FREDERICK DUCANE GODMAN, 1834—1919.

THE death of a man who has taken such an active and distinguished part in zoology for so many years, is a loss to science, which all who knew him must deplore.

Godman was born in January, 1834, the third son of Joseph Godman of Park Hatch, Godalming, who as a partner in the firm of Whitbread and Co. was able to leave him an ample fortune. As a boy at Eton, where he went in 1844, he was delicate in health, and after three years was removed and continued his education under private tutors. Before he went to Cambridge he was sent on a tour to the Mediterranean and showed his independence by refusing to go home with his tutor, who wished to return to England by a vessel sailing six hours after their arrival at Constantinople. He made the acquaintance of some English travellers, and went on a trip with them to the Black Sea and to Sebastopol, about which city he was called on later to give some information to the War Office before the Crimean War. He returned to the Crimea in a yacht in 1855 to visit his brother in the 5th Dragoon Guards—Captain, afterwards General Godman—who was serving in the war. He then witnessed the storming of the Rifle Pits, the capture of the Mamelon, and the entrance of the Allied fleets into Besika Bay.

In 1858 he entered Trinity College, Cambridge, where he first met Osbert Salvin and the late Prof. Edward Newton, of Magdalene College. This association was without doubt the main influence which determined his future career; for without it Godman, whose love of sport was as strong as it is in the sons of most English country gentlemen, might have become, as many do under similar conditions, a worthy member of his native county society, a master of hounds, a respected magistrate, or even an M.P. But the love of Nature which was born in him was guided and increased by the formation whilst he was at Cambridge of the British Ornithologists' Union, and by close association with the original founders; amongst whom Alfred Newton, P. L. Sclater, and Canon Tristram, all members of this society, did much to train the young ornithologists of the day to a high standard of accurate observation, correct nomenclature, and scientific thoroughness, which has always distinguished the 'Ibis' and its contributors. It may be said that ornithology is not a science which can conduce very much to the welfare of the world, but Godman's love of natural history was not confined to birds, for he also took an active interest in plants and insects, and became President of the Royal Entomological Society in 1892. His Presidential Address in 1893 ('Ent. Soc. Trans.,' 1892, vol. 46), contains some observations which may be profitably read at the present time by those who are inclined to minimise the utility of systematic collecting. Boys with a love of nature, who possess the hunting instinct which usually accompanies it, habitually begin with birds and butterflies. Most of them under the influence of classical teachers are taught to consider such pursuits as trivial, and when they grow older they drop them as being boyish. But as Godman pointed out in his address, when speaking of the late H. W. Bates, F.R.S., the combination of qualities necessary to make a good traveller, a good collector, and a good observer, all of which Godman himself possessed to a high degree, "are of special value at the present day, when it is the practice of some to extol certain branches of our subject, and to decry others; to exalt generalisations, and to depreciate the drier and less attractive labours of the systematist." And as I tried to point out in my own Presidential Address to the same Society in 1895, this tendency, which seems to be increasing amongst botanists as well as zoologists, has a bad influence on many young men who are encouraged by the example of their teachers to neglect that exact knowledge of the objects they study, which can only be attained by collecting. Generalisations, so often based on imperfect knowledge of classification and geographical distribution, can only be attempted with success by the few men whose brain power is much above the average. Godman was not such a man, and never pretended to be, but by his own work, life and example, he made it possible for others to do such work at a future period, by providing and describing the material on which alone they can safely rely. It is certain that an accurate knowledge of the obscure and difficult orders of insects, which have in the past materially obstructed the progress of colonisation in our tropical possessions and colonies, can only be obtained by the encouragement of such work as has been done and is being done by men, who have no professional scientific training, but have devoted themselves to science in their thirst for knowledge.

The close friendship which Godman formed at Cambridge with Salvin has been well described by the late Prof. Newton in his obituary notice of the latter (*cf.* 'Roy. Soc. Proc.,' vol. 44, March, 1899); this developed later into a lifelong scientific partnership, which is unique in the annals of zoological science, and enabled them to do together what neither man could have done alone. For though it was at Godman's expense that the collections were acquired, they were, together with the library, held in common, and they continued their close association and intimate friendship after Salvin's marriage in 1865 and until his death in 1898.

Though Salvin had probably a deeper insight into the difficult questions of classification, and was able in many ways to inspire Godman's work, yet no one could ever say that either partner took a lead, or that their respective shares in their joint work were not carried on without the least shade of difference or dissent.

On leaving Cambridge Godman began his ornithological travels. His first expedition was with his brother Percy (now the sole survivor of the original members of the British Ornithological Union) to the north of Norway, when he visited John Wolley, the greatest oologist of his day, in Lapland, and walked across that country on his way to Sweden and Russia. An account of this journey was Godman's first essay in publication and appeared in the 'Ibis' for 1861. His next journey, in 1861, was with Salvin to Guatemala, where the latter had already made large collections of birds and plants in 1857 and 1859, their object being to investigate the problems of geographical distribution and evolution, in which Darwin's 'Origin of Species' had aroused their interest. This journey, which lasted for some time, took them through many remote districts then hardly known to travellers, and the rich collections they formed were the nucleus of what afterwards became the most complete collection of birds, mammals, and insects ever formed in Central America, of which I shall say more later. In 1865 Godman, accompanied by his brother, Capt. Temple Godman and an entomologist, visited the Azores, to investigate the fauna and flora of that group of islands, which were then little known and specially interesting on account of the light which they throw on geographical distribution. He visited all the islands except Santa Maria, and brought back a large collection, which he afterwards described in a book on the Azores, published in 1870.

In 1871 he went to the Canaries and Madeira with the same object, and published an account of this in the 'Ibis' in 1872, which resulted in an interesting correspondence with Darwin.* In 1876 he and Salvin formed the idea of publishing the great work 'Biologia Centrali-Americana,' which gradually developed into the largest series of the sort that has ever been privately published at the expense of one man, and which occupied the authors for the rest of their respective lives. The collections which were gradually brought together for this work, were first stored in Salvin's house in London, and when he was appointed Curator of the Strickland collection of birds at Cambridge, Godman took a house in Tenterden Street, where until 1878 the joint museum and library were housed. Here the late Lord Lilford, President of the British Ornithologists' Union, also had rooms, and the house became a centre of ornithological society and fellowship. Later on, the collections were removed to Chandos Street, where they remained till after Salvin's death in 1898. The two friends had previously determined to present the whole of their collections to the British Museum of Natural History, and, with his usual disinterestedness, Godman decided that they should be incorporated in the National Collection as soon as they had been worked out. For this purpose he employed his own skilled workers, and at the time of his death the great task of incorporation was almost concluded. The great Henshaw collection of birds of the United States was added, and several private collectors had been employed for many years in various localities in Mexico, and in Central and South America, so that the mass of material was enormous. The number of bird-skins alone eventually handed over amounted to no less than 520,000, the largest single donation ever made to the Museum of Natural History.

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* Probably in connection with the boulders in Tercho Ste. Maria (see 'Origin of Species,' p. 393), Godman maintaining that the islands were volcanic and the boulders merely ballast.

The original plan of the 'Biologia' was to publish six quarto parts annually, made up of various subjects, with six coloured plates in each, so paged and numbered that they might when complete be broken up and bound in their respective volumes. The work gradually grew until, when finished, it consisted of 63 quarto volumes; of which 1 forms the Introduction, 51 relate to Zoology, 5 to Botany, and 6 to Archæology. Of these, three volumes on Birds and three on Butterflies were the sole work of Godman and Salvin, whilst those on other orders of insects were the work of various specialists, those on Botany by Mr. Hemsley, and those on Archæology by Mr. A. P. Maudslay. They are illustrated by no less than 1677 plates, of which over 900 are coloured; and the total number of species described is 50,263, of which more than 19,000 were new.

Neither to Godman nor Salvin was granted the health and strength to complete the work as they had originally intended, but to the former additional years gave time to bring many descriptions to an end, and with the assistance of the best zoologists of the day a summing up of each subject was made, accompanied by maps specially drawn for the purpose. The larger intention of publishing the deductions arrived at during such an extensive work would have occupied years, and, with failing health, Godman wisely elected to offer to the world a book, already more vast and complete in detail than anything ever yet privately published, stating, as he did in the preface, that in laying the foundations, he trusted that others might develop the work.

In 1880, Godman accompanied me on a short trip to India, during which we visited the North-west Himalayas and Sikkim, advancing into the interior as far as the snow would allow. The wonderful views of Kinchinjunga from a point very near its base, and of Mount Everest by moonlight from an elevation of 13,000 feet impressed Godman deeply, and great traveller as he was, he always referred with delight to that splendid scenery. Here the first symptoms of a slight weakness of the heart, which afterwards showed itself at lower elevations, became evident, and on one occasion this overcame Godman so seriously that I was for a time very anxious about his recovery; but he was so active and keen both in sport and collecting, that he did not hesitate afterwards to undertake a long and rather dangerous journey through the fever-stricken jungles of the Bhotan Duars to get a very rare and remarkable butterfly which had then only once been taken near Buxa. In 1885 he was advised to spend the winter in a warmer climate than England, and went to Mexico in the autumn, accompanied by two ornithological and two entomological collectors who worked in parts of the country where no previous collections had been made. He visited the celebrated ruins of Yucatan, which have been so admirably figured and described in the archæological part of the 'Biologia' by Maudslay, and when I joined him in the winter we visited Orizaba and ascended the volcano of Popocatapetl, where we made large collections, returning *viâ* California, visiting the Yosemite Valley and afterwards the Yellowstone National Park.

About this time Godman became very interested in plants, and at his home, near Horsham, he formed a good collection of orchids, and one of the best of Alpine plants, growing them with great success, and gradually laying out one of the most beautiful and interesting gardens in the south of England. Rhododendrons, which the soil suits admirably, also interested him very much. In his garden he was always careful to have the numerous rare plants correctly named, and in his later years derived the greatest pleasure from their cultivation and from sharing them with numerous visitors. Farming and building occupied his attention, and his practical knowledge was the admiration of his workpeople.

Godman's first wife, the daughter of the late J. H. Elwes, of Colesborne, Gloucestershire, died in 1875 without issue. In 1891 he married Alice Mary, the daughter of the late Major Percy Chaplin, who, with two daughters, survives him. Together with his wife, he visited the West Indies, Egypt, South Africa, Rhodesia, as far as the Victoria Falls, etc. On a second trip to Egypt they journeyed to Khartoum and up the White Nile as far as Goz Abu Guma, and later joined forces with Dr. Andrews in the excavation of fossil animals, including the Arsinotherium, in the desert of the Fayum.

Possessed of remarkable powers of observation and an intense love of nature, Godman succeeded in all that he undertook. His collection of Persian, Rhodian, Damascus, and Hispano-Mauresque ware is the finest in existence, and in other arts as well he was no mean connoisseur, while his readiness to share his treasures was shown by the frequency of his loans to the South Kensington Museum and other art galleries. His taste for Geology was very strong, and in later years this subject and that of Palæontology almost rivalled his lifelong devotion to Zoology; the recent discoveries at Piltdown were immediately visited, in company with his friend Godwin-Austen, and the work that followed afforded him a lasting interest.

Highly gifted as he was, no man was more modest, and a shyness amounting almost to a horror of public speaking made Godman avoid publicity; but in his own house few could equal him as a host, and his fine nature, vast stores of learning, and varied abilities had full scope, and in the hundreds of letters of condolence received from men of all ranks and shades of opinion there is a unanimous expression of affectionate admiration for one who exercised the charm of a well-nigh perfect nature combined with a wide understanding.

In 1882 Godman had been elected to the Royal Society, and in reviewing the life of this remarkable man, I cannot but feel that it is a very great misfortune that so few men of the Godman type are now able to attain the honour of Fellowship in this great Society. If one looks through the list of candidates of recent years it will be seen that country gentlemen, who have trained themselves in scientific work and who could be encouraged to do a great deal for science, are conspicuous by their absence. There seems to be a tendency to limit the elections into the Society to men who, making their living by the teaching or practice of some branch of science, are able to specialise sufficiently, in order to publish what is now considered to be the necessary amount of original work. I feel sure that the Society would gain if more men of the Godman type could be led to believe that the Fellowship of the Society is not closed to them.

As a Trustee of the British Museum, Godman probably had a wider knowledge of the needs and working, certainly in the Natural History part, than anyone else, and he spared no time or trouble in keeping himself in close touch with it. He was keenly alive to the disadvantages of the Civil Service system when applied to such appointments, holding that work was better done and the interests of the Nation best served, by the payment of good salaries rather than by advancement by seniority and deferred pay in the shape of a pension. He never ceased to deplore the loss of valuable time incurred by the amount of clerical work expected of heads of Departments, whose time for scientific work was correspondingly decreased.

Godman was devoted to sport, especially hunting, fishing, and deer-stalking, and in his youth to rowing and swimming. Two of his feats in this direction are worthy of mention, namely, the crossing of the Hellespont and of the Menai Straits, both against strong currents and both entirely unpremeditated excursions. For many years he rented deer forests in the Highlands, where he was as much beloved as he was by his own employees at home. Even when his health began to fail, and his physical powers had decreased for some two or three years before his death, his interest in science and in everything around him never failed, his unvarying good temper and kindness of disposition never left him. He died in London quietly from heart failure, after a short illness at his house in Pont Street, and was buried at Cowfold, in Sussex, where his funeral was attended by a great number of people of all classes who had known and loved him.

Godman was an active member of many societies; he had been Vice-President and was long on the Council of the Zoological Society, a Past-President of the Entomological Society, of the British Ornithologists' Union, Gold Medallist of the Linnæan Society in 1918, a Trustee of the British Museum, and a D.C.L. of Oxford University. He was also a Fellow of the Society of Antiquaries, of the Geological and Royal Geographical Societies.

The British Ornithologists' Union have decided to commemorate him, in conjunction with Salvin, by the institution of a Godman-Salvin Gold Medal, to be given by the Union for specially good work in Ornithology; and another committee has been formed at the British Museum of Natural History to enable members of other societies to present a memorial to that institution in the shape of a bronze portrait-plaque of the two friends, and a fund for travelling and collecting, to be called the Godman Exploration Fund.

H. J. E.

JAMES WILLIAM HELENUS TRAIL, 1851–1919.

James William Helenus Trail was born at Birsay, in Orkney, on March 4th, 1851. He was the youngest son of the Very Rev. Samuel Trail, minister of Birsay and Harray, 1843–67; Professor of Systematic Theology, University of Aberdeen, 1867–87; Moderator of the General Assembly of the Church of Scotland in 1874.

After a sound preparatory education at home, Trail was sent to Mr. Tulloch's academy, then a good private school in Aberdeen. From this he was transferred, in 1865, to the Grammar School of Old Aberdeen, where he was subjected to the intensive classical drill then deemed essential before matriculation. In Trail's case this hard discipline, in place of increasing, had the effect of destroying any enthusiasm he may ever have shown for classical study. As a child he had been addicted to the observation and collection of natural objects. While at school under Mr. Tulloch this predilection had been encouraged and directed into methodical channels. Even at the Grammar School his favourite pursuits occupied his scanty leisure. The habit continued after he entered the University in 1866. Though a good student in all the arts departments, he did not seek distinction in classics, in mathematics, or in philosophy. He concentrated his attention on the subjects in the Natural Science Department, in which he obtained "highest honours" when he graduated M.A. in 1870.

Entering the medical faculty, which at that time provided the only avenue the University could offer to those anxious to adopt a scientific career, Trail was not particularly attracted by his purely professional studies. On the other hand, he served, during the years 1870–73, as assistant to the professors of botany and chemistry and to the curator of the zoological museum. In 1873 he gave further proof of his attitude towards medicine by abandoning his studies altogether, in order to take advantage of an opportunity of learning at first hand what a tropical fauna and flora are like. He accepted the position of naturalist to a South American exploring expedition, and in this capacity was able to travel upwards of 16,000 miles on the Amazon and its northern tributaries, making ample notes of his observations and securing large zoological and botanical collections.

Returning in 1875, Trail resumed his medical studies and at the same time set about the arrangement of his material and the publication of some of his results. The value of his material so impressed those competent to judge that in December, 1875, Trail was elected to the Linnean Society. His medical studies gave him little trouble and in 1876 he graduated M.B. with "highest academical honours." The thoroughness and ability displayed in working out his collections led to his selection, towards the end of 1876, to fill the post of Government botanist in British Guiana. Early in 1877, however, when Trail was about to leave, the Professor of Botany at Aberdeen, the late

Dr. G. Dickie, F.R.S., was compelled, on the advice of his physician, to tender his resignation. The Crown appointed Trail to the vacant chair, the duties of which he took up in May, 1877.

When Trail was appointed it was remarked in some quarters that the Crown had chosen a zoologist to fill a botanical chair. The authors of this comment could justify it by citing the circumstance that of the 30 odd contributions to natural knowledge made by Trail during 1870–76, 14 relate to purely zoological observations. The comment was nevertheless a superficial one, for a dozen of the remaining articles were contributions to the study of galls, a subject of equal interest to zoologists and botanists, while of the four purely botanical contributions two are important additions to the history of palms. What had really happened was that the Crown took the opportunity —even then far from common, and to-day, unfortunately, rarer still—of appointing to a biological chair an incumbent who had already given signal proof that he was an accomplished naturalist, endowed with a marked capacity for exact and patient observation, and an extensive acquaintance with the morphological features and the bionomic relationships both of plants and of animals. That Trail was as competent to conduct a zoological as a botanical class he soon had an opportunity of showing. The chair of zoology in the University became vacant in 1878, and the professor appointed by the Crown was prevented for a year from taking up the duties. At the request of his University, Trail taught the zoology class during 1878–79 with as much success as attended his conduct of the botany one. As events were to prove, one of the factors that made Trail so effective and useful as a teacher was the unconscious ease with which he could apply his wide and exact zoological knowledge to the explanation of facts and phenomena connected with plant life.

When Trail took up the duties of his chair the equipment of the botanical department was extremely defective. This was neither due to neglect on the part of the eminent algologist whom he succeeded nor owing to any want of sympathy on that of the University authorities. The opportunity which the task of teaching two classes afforded, enabled Trail to think out a scheme whereby these defects might be remedied with due regard to the needs of his chair on the one hand, and to the economic condition of the University on the other. By 1879, the year in which he proceeded to the degree of M.D., Trail had begun to carry out his designs. With patient but unflagging courage and energy he kept to his purpose, with the result that he has left for his successor a well-equipped and thoroughly modern botanical department, with an excellent teaching museum, well-furnished laboratories, and an admirable botanical garden.

While immersed in this task, Trail was given an opportunity, in 1883, of accepting the leadership of an important exploring expedition to tropical East Africa. He undertook the duty subject to the condition that he be shown the consideration extended to the professor of zoology in 1878, and be excused the responsibility of nominating the substitute required to conduct his class during his absence. This, fortunately for his department, the authorities concerned were unable to arrange. Trail, however, only escaped one duty in order to undertake another. From 1871 onwards his communications on the subject of galls had been published in the 'Scottish Naturalist,' and had led to the establishment of a warm friendship between himself and the editor, Dr. Francis Buchanan White. In 1883 White resolved to relinquish the editorship and persuaded Trail to take it up. Trail edited the journal from 1884 until, in 1892, it was merged in the 'Annals of Scottish Natural History,' when he undertook the botanical editorship of the new serial. He fulfilled these duties during the 20 years that the 'Annals' were published.

In 1886 Trail took a prominent part in the foundation of the Aberdeen Working Men's Natural History Society. The success of this organisation he did much to ensure by often guiding its discussions and sometimes leading its excursions. The members repaid his assistance by appointing him year by year to serve as their president. By 1889 his work as a student of galls had secured for him a European reputation, and led to his election in that year to the Zoologisch-botanische Gesellschaft of Vienna.

The energy and tact displayed by Trail in the organisation and development of his own department led to his being invited to undertake a new and important duty. In 1891 the University Commissioners called for a report from the library committee on the condition of the library. Trail, who had been a member of this committee since 1877, was unanimously requested to accept the posts of curator of the library and chairman of the committee, and to draft the necessary report. How well he accomplished his task may be gathered from the circumstance that he was thereafter re-elected to both posts every year. The present condition and organisation of the library owe much to his foresight and care. The following year saw a further addition to his academic duties. A faculty of science was established in the University, and in 1892 he was appointed its dean. But these added tasks in no way impaired his efficiency as a teacher or his activity as an observer. His attainments in the latter capacity were recognised by his election to the Royal Society in 1893 and to the Deutsche-botanische Gesellschaft in 1894.

The year 1895 brought Trail a new engagement. In 1894 his friend Buchanan White had died. This able naturalist, like Trail himself, was as competent a zoologist as he was a botanist. The sympathies and interests of the two were identical, their comradeship had long been close, and when it was represented to Trail that it was desirable to prepare for publication the unfinished manuscript of White's 'Flora of Perthshire,' he undertook the work without hesitation. It proved a heavy obligation, but, with the help of mutual friends of the author and the editor, the task was completed in 1898. This, however, was by no means the only extra-academic duty undertaken by Trail during the same period. His business capacity was as fully appreciated outside the University as within its walls, and his help was eagerly sought. Taking no part in political or municipal affairs, he nevertheless felt it his duty to respond to appeals connected with the advancement of education, especially scientific education. This led to his serving as the chairman of more than one education committee, and as a governor of various educational trusts. It also induced him to take a leading part in the movement which culminated in 1903 in the foundation of a College of Agriculture, and to accept the presidency of the Buchan Field Club, which he demitted in 1904. He was equally active in the movement which led to the foundation in the University of a Forestry lectureship, and he showed his readiness to further the cause of science generally by serving as president of the botanical section of the British Association at the 80th meeting, held in 1910.

The last ten years of Trail's scientific activity were devoted to the accumulation and arrangement of data for a 'Flora of North-Eastern Scotland,' on a scale wider and more comprehensive than anything of the kind hitherto attempted. It is a source of deep regret to his friends that his death should have placed his own material for this work in the jeopardy from which he was able to rescue that of the late Dr. Buchanan White. The strain induced by war conditions had the effect of preventing, after 1913, the annual visits, spent in working at Kew and the British Museum, to which he was wont to look forward. Otherwise the difficulties of the time did not appear to affect his life or curtail his energy. With the cessation of hostilities he had to face that sudden increase in the number of students which has been the common experience of seats of learning. His class, in 1919, had to be divided into two moieties, and with characteristic self-sacrifice, he resolved to give as much personal attention to each pupil as was his practice in former years. Whether this doubling of the hours devoted to the work of his class played any part in inducing or aggravating the insidious malady—a duodenal ulcer—which ended his career is not clear. The presence of his ailment was unsuspected until perforation took place. Removed to a nursing home in Aberdeen, he stood well the surgical interference that was called for, and for a time hopes of recovery were entertained. But disquieting symptoms supervened, his strength failed, and he died on September 18, 1919, in his 69th year.

Though by no means a fluent speaker, Trail was a clear and convincing teacher. His statements, while never dogmatic, were always precise, and were conveyed in language chosen with care. The matter discussed was used primarily as a means of education; the imparting of instruction, which was ample, appeared to be incidental to his main purpose. This was particularly evident in his practical classes, where he used his subject as a means of developing the faculty of observation and exercising the reasoning capacity of his pupils. Ever ready with effective assistance in overcoming difficulties connected with technique, formal demonstration was skilfully avoided, and the solution of the problem set was left to the unaided efforts of the student. The same purpose underlay his methods as the leader of class excursions, during which he was able, while retaining the esteem and respect of his pupils, to convince them of the essential solidarity of the interests of teacher and taught. Outside his class he did much during many years for successive groups of undergraduates by the measures he adopted to increase the efficiency of the University battery. He joined the corps himself with a subaltern commission, and by assiduous study and practice made himself an efficient officer, earning his eventual promotion to field rank and the command of the corps solely by merit and without reference to his position in the University. The result was that the endeavour to obtain efficiency, which he expected on the part of others, was readily and successfully made.

The high sense of duty which led Trail to lend his organising powers to the University and the community at large cannot be looked upon as a matter for regret. The services he so ungrudgingly rendered were in every instance devoted to the causes of higher education and of science. There was, however, an inevitable consequence of this manifestation of civic virtue which must be regarded as unfortunate. The notes and papers, some 250 in number, in which Trail's contributions to natural knowledge have been published are of such a quality as to justify our regret that they are not more numerous Those who had the privilege of Trail's personal acquaintance are aware that these published notes represent but a small fraction of the observations carefully recorded by him during his scientific career. His results were at all times freely at the disposal of anyone who cared to consult him. It is among those who owe him most gratitude for help thus rendered that the greatest regret is felt that so little of the wide and exact information secured by Trail as the result of his own observation is now available for use by others.

To the furtherance of scientific education and research Trail devoted means as well as time. In memory of his mother he founded in the University a fund to be employed in assisting undergraduates in any of the faculties who may display approved proficiency in Natural History studies. On completing 25 years' continuous service as curator of the University library he founded another fund, to be used, in supplement of regular grants from the University purse, for the purchase of scientific books. To the Linnean Society he committed the management of a third fund, to reward special research. But these recorded gifts bear even less relationship to Trail's constant but unobtrusive benevolence than his published contributions to natural knowledge bear to the vast store of scientific facts, peculiar to himself, secured by patient observation in many fields of Natural History.

Quiet in manner and naturally reserved, Trail did not often intervene in discussion. When, on being invited, he did speak he was listened to with attention, not only because of his wide knowledge, but because of his manifest sincerity and his balanced judgment. In Trail those who had the privilege of his friendship have lost a wise and kindly counsellor; natural science has lost a distinguished worker in a field where much remains to be done and the labourers are far too few.

D. P.

EDWARD SAUNDERS, 1848—1910.

Edward Saunders, who died February 6, 1910, at Bognor, was born March 22, 1848, at East Hill, Wandsworth—the youngest of the seven children of William Wilson Saunders, F.R.S., for eleven years Treasurer of the Linnean Society, and well known for his immense Entomological and Horticultural Collections and for his wide interest in science. His house, Hillfield, Reigate, to which he moved from Wandsworth in 1857, formed a great centre where scientific friends used to gather to study the collections and to meet one another. Nor was the convivial side by any means wanting, as may be inferred from a memory of my friend Prof. Clifton who happened to meet one of the guests on Reigate platform. "I had a great honour last night," said he in a manner half-sly, half-affected, "I was put to bed by Mr. Westwood."

It was primarily for the Wilson Saunders collections that Alfred Russel Wallace set out on his famous expedition to the Malay Archipelago.

Educated at home, although he went for a time to a day school at Reigate, constantly in touch with the great collections, and meeting those who came to see them, always encouraged by his father and stimulated by his brother George, his sister, and his half-brother William Frederick, the naturalist members of the family, Edward Saunders grew up in conditions certain to bring out and develop to the full any taste for natural history that he possessed. But after all the essential thing was the innate possession, together with the critical faculty which made it of value. Conditions may evoke but they certainly do not create the qualities which made him, not yet thirteen, the predominant partner in preparing, in conjunction with his brother George, a list of the Mollusca of the Reigate District, published in February, 1861 (3).

His sister, Mrs. T. R. R. Stebbing, recalls memories of his early interests:

"We spent happy, busy, sheltered lives, without much of the style of education of the present day, but with much to turn our thoughts towards science. My father was a very busy man, engaged at Lloyd's as an underwriter all day, and full of his Natural History work and his garden when at home, but always ready to encourage his children in collecting.

"My youngest brother Edward and I, were always keenly fond of plants, and when we were quite little, father would give us a penny a dozen for pressed plants, and three pence a dozen if we wrote in their Latin names, which he would tell us. We used to collect them on Wandsworth Common, and we left Wandsworth for Reigate in 1857, when Edward was nine years old.

"At Reigate he soon got interested in English land- and fresh-water shells, and in birds and birds' eggs. I remember one of the first times that we were allowed to take a walk by ourselves, going to a chemist at Redhill for

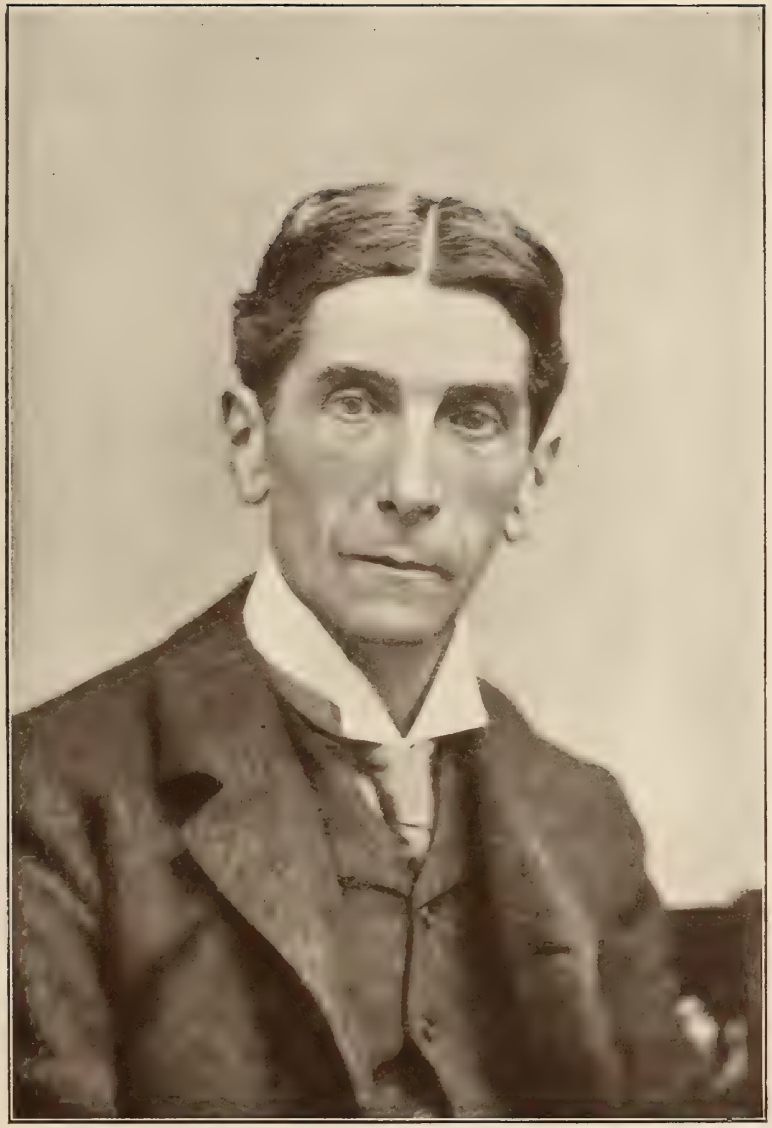

Photo by Elliott & Fry, Ltd., 1905.

Edward Saunders

arsenical soap for preparing bird-skins, and how offended my brother was at the chemist objecting to giving it to us, as we were too young; but we got it in the end. Edward was then (about 1859) going to a small private school each day, and as time at home was limited we used to get up at 5 o'clock each morning to divide a collection of dried plants from Scotland that my father had given to us. Edward was very keen on grasses and he made a good collection of them. It was about this time that he began to be specially interested in Entomology."

Edward Saunders travelled abroad a good deal, especially in the early part of his life. His round of the foreign museums, in order to study the types of *Buprestidœ,* was far from being, as has been stated (1), his only visit to the Continent.

It is probable that Wilson Saunders' many interests interfered with the claims of marine insurance; at any rate, the collapse came in 1873, and the great collections had to be sold. It was fortunately possible to preserve some of them from being broken up. Of these, the greater part are at Oxford, although important groups are in the British Museum (N.H.) and in the collection of Mr. J. J. Joicey. Edward Saunders, who had entered his father's office in 1865, had henceforth to depend upon his own exertions, and could only devote himself to science in his scanty leisure.

A paper on "Coleoptera at Lowestoft," published when he was 16, in the first volume of the 'Entomologists' Monthly Magazine'—the journal he was to edit for the last 30 years of his life—marked the beginning of his life-work in insects. He soon began to specialise in the *Buprestidœ,* continuing to publish papers on the family up to 1874, the classical 'Catalogus Buprestidarum Synonymicus et Systematicus' appearing in 1871. Before he had finished with this group, he was bringing out notes on the British Hemiptera, followed in 1875-6 by the 'Synopsis of the British Hemiptera-Heteroptera,' and in 1892 by his volume on the same subject. While still attacking these two distinct and widely separated groups, he began on, what was destined to become the chief work of his life, the Aculeate Hymenoptera. It cannot be doubted that he was specially drawn to these insects, for the reason he gave to his friend, the Rev. F. D. Morice (1), as well as to the present writer, viz., the importance, and, indeed, the necessity for the study of *structure* in the determination of the species. And it was the surprising possibilities of the modification of structure which prompted a vividly remembered saying of his. We were glancing through the boxes of his father's collection at Oxford, and came upon a male of *Synagris cornuta,* L., from Fernando Po, a specimen in which the enormous horn-like outgrowth, from the base of the mandible, found in many males of this wasp was developed to an extraordinary degree. "Why," said Edward Saunders, "it is a biological education to look at that insect!" ('Report Brit. Assoc.,' 1913, p. 512).

Then, too, there was the opportunity afforded by the abundant material at Reigate, and in the Grecian Hymenoptera, many of which were given to him, collected by his father's first cousin, Sir Sydney Smith Saunders,* Consul-General in Albania. He took the greatest interest in Sir Sydney's collection at Oxford, and one of his last pieces of work was a careful revision of the whole of the determinations. It is unnecessary here to do more than refer to the great series of papers on the Palæarctic Aculeates. Of "his grand work, 'The Hymenoptera-Aculeata of the British Isles' (1896)," it has been well said (1) that it "is one of the few without which no serious Hymenopterist thinks his working-library complete, and that its merits have been acknowledged in the warmest terms by everyone at home or abroad who is competent to form an opinion upon it."

Edward Saunders realised the importance of writing simply so as to stimulate the interest of beginners, and with this object published in 1908 an introduction to the study of the British Wild Bees, Wasps, Ants, etc.

It was a pleasure to his friends to arrange that his collections are bestowed, as he would have wished, where they are accessible to the student—the Aculeates in the British Museum, the Hemiptera in the Hope Department of the Oxford University Museum.

He joined the Entomological Society in 1865, was Treasurer 1880–1890, and a Vice-President in five sessions, the last in 1907. It was a great disappointment to his friends, and indeed to the whole Society, that he would never consent to be nominated for the office of President—an election that would have been received with pleasure and pride. He felt that, in the state of his health, it was imprudent to bind himself to attend the evening meetings, although the present writer was led by some words used by Saunders, when he was being strongly urged to accept the nomination, to think that he cherished the vain hope that he might at some future time become stronger and be able to consent.

He became F.L.S. in 1869, F.R.S. in 1905.

It was only by a wonderful power of concentration, the rigid economy of time, and the utmost use of his limited strength that one, whose daylight hours were nearly all of them occupied with the cares of business in the Royal Exchange, could do so much minute and accurate work and be able to give such constant and most efficient help to less experienced students. He must have had great insight into potential qualities, for one at least of his correspondents was in the schoolboy stage of his career as a Hymenopterist when Edward Saunders began to devote to him a considerable share of his scanty time (p. xvi).

With this power of concentration he did not require to be alone, but could work surrounded by his family, although it is a mistake to suppose that he had no study of his own (1). He was, however, rather cramped for want of space, for I remember his special request that specimens should be sent to him in small boxes, and not in the original large and heavy ones which belonged to his father and cousin.

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* Died April 15, 1884. 'E. M. M.,' 1883–4, p. 278; 'Entomologist,' 1884, p. 117.

His day was planned as follows: He would sometimes work before breakfast, and always afterwards until it was time to start for Lloyd's, and then many of his letters were written in the train. He was generally back again by 5.30, and after a cup of tea gave the rest of the evening, before and after dinner, to his scientific work. His holidays, too, were entirely devoted to science, including here the out-of-door collecting and observing in which he was highly successful.

Edward Saunders was acutely critical, and at the same time sympathetic, kind-hearted, and generous—an uncommon combination when the qualities are developed to the high level they reached in him. He was deeply religious. In good health he had unfailing spirits, and was quick to see the comic side of things. He loved pictures, and music even more, and for a good concert he would even lay aside his scientific work. In youth he was fond of games and sport, but sold his favourite gun in order to buy a good microscope (1). Later in life he played croquet well, and still later lawn tennis, until he was obliged by illness to give up all except the gentlest form of exercise.

One of his sons has recalled the dominant impressions of his father's personality:—

"The point that struck me most about my father was his very deep religious feeling. It was not quite the ordinary everyday religion, but it permeated his business and his whole life. One of my earliest recollections of this was once walking across Horsell Common with him on a Sunday, and I picked up a Carabid beetle which he had never taken in the district before—I forget which one it was but I remember it was very green—and he would not let me take it as it was Sunday. I remember being very sad as I knew he wanted it. Next day he sent me out to see if I could find it again, but, of course, it had gone! He would never collect any living thing on a Sunday.

"In public life he never shone as he was too retiring, but he did a great deal of work quietly and unknown to most people. He was not content until a Cottage Hospital was made for Woking. He would superintend choir practices at the Church because the singing was not up to his ideas of worship. He was a great opponent of dull services in the very Evangelical Church at Woking. The parson and curates loved him, although he fought with them. They also nearly always gave way to him in the end."

Edward Saunders lived successively at Reigate, Wandsworth, and Bromley, settling finally at Woking in 1887. In 1872 he married Miss Mary Agnes Brown, of Wandsworth, who survives him, with eight of their twelve children. One son, Charles, after being wounded at Loos, was killed on August 18, 1916.

To his children he was both kind and generous, although the standard he unconsciously set was sometimes rather alarming; for remembering his own youth, but with characteristic modesty unable to recognise its exceptional promise and performance, he was led to expect a somewhat discouraging level of scientific attainment at an early age.

A picture of Edward Saunders, as a young man, is recalled by his friend, Mr. G. A. J. Rothney: "I can see him now as he was in 1868 to 1870, looking so very young and boyish, but standing up and reading learned papers at the Entomological Society on the Buprestidæ, listened to and holding his own with the giants of those days—Westwood, Lubbock, Bates, Wallace, McLachlan, Stainton, and Frederick Smith."

For many years before his death, Edward Saunders had suffered from lung trouble, and had been gradually losing strength, and when, in March, 1909, he was seized with influenza he never fully recovered. "At the last there was extreme weakness, but happily no pain whatever, and his interest in scientific matters was keen and bright up to the very end." (1)

I am glad to be able to quote, in concluding this brief memoir, the impressions of two Hymenopterist friends, one of about his own age, the other much younger. The abiding memories of both are the same as my own and dwell on his unbounded kindness and generosity to those who needed his scientific help.

Mr. G. A. James Rothney remembers that Edward Saunders was glad to see a fellow naturalist, even when he called at Lloyd's, and brought him out of that busy room to talk about ants and bees. "I often thought," he writes, "that he must have inwardly cursed me, when the red-coated official called his name, and he hurried out to find no business but an ant-man who wanted help; but he never showed it, and was always smiling and genial in his welcome. Like Frederick Smith, he had the gift of rapidly sketching a character or feature of a specimen on the back of an envelope or any scrap of paper. In his home it was not difficult to see that he was loved by all, and was the friend and comrade of his sons as well as their father. He was a good man in the highest sense, and in everything the soul of honour. I never came away from a visit without feeling in every way the better for it. I had a great admiration and respect, and I may say love for him. He was the kindest helper and friend that any one could have."

Dr. R. C. L. Perkins writes:—

"It is rather remarkable that I never actually met Edward Saunders, though I corresponded with him continually for over 20 years! Once when I had arranged to meet him, I rather unexpectedly and hurriedly left again for Hawaii. Nevertheless I seem to have known him very well, for at some periods I think we must have written to each other almost weekly or at least once a fortnight.

"When I think of Edward Saunders it is always his kindness and generosity to beginners that first comes into my mind. When I first began to collect and study Hymenoptera I was only a schoolboy, well acquainted already with Lepidoptera; but to a Lepidopterist the change from his own group to such different things as bees and wasps naturally presents considerable difficulties at first. As it happened several of Saunders' nephews were at school with me at Merchant Taylors', and one of them kindly obtained the loan of a book or two for me. In this way I got into correspondence with him. He not only named many specimens for me, as a beginner, but sent me a copy of his 'Synopsis' and gave me many accurately named specimens of his own to facilitate study. I should think no man was ever more generous—both in giving specimens and assistance in naming. The latter must have taken up an enormous amount of the time he could devote to Entomology, for I know that some Hymenopterists, who had not the gift of appreciating difficult specific characters, were in the habit of sending him the same species, year after year, for the purpose of getting these named wholesale.

"So far as his work on Hymenoptera is concerned, though this was not very extensive, it was of excellent quality. In my opinion none of our Hymenopterists of the past can be considered in the same class, excepting only Haliday. With the publication of the 'Synopsis' and some of the papers of nearly the same date, our knowledge of that branch of British Hymenopterology, which had, since Kirby's 'Monographia,' lagged behind the best continental work, became comparable with the very best of the latter.

"Systematic entomologists are, or should be, notorious for their gross carelessness, but I should certainly consider Saunders an exception, or at least as a very slight offender, though I find that more than once in his letters to me he speaks of his great fault—'carelessness.' Compared with most systematists he certainly had nothing to reproach himself with in this respect. There is no doubt that he was a very clever and shrewd Hymenopterist—excellent both in the field and study alike. But his encouragement of the beginner and the help he was so ready to give to any one who asked it, however much it must have encroached upon his own time, always returns to my mind, when I think of him."

The writer is indebted to the following obituary notices of Edward Saunders, as also to members of his family, to Dr. R. C. L. Perkins, and Mr. Rothney:—

(1) 'The Entomologist's Monthly Magazine,' 2nd series, vol. xxi, March, 1910, p. 49, with a portrait, by the Rev. F. D. Morice.

(2) 'The British Bee Journal,' April 7, 1910, p. 135, by F. W. L. Sladen.

(3) 'The Proceedings of the Linnean Society of London,' Session 122, 1909–10, p. 94, by the Rev. T. R. R. Stebbing, F.R.S.

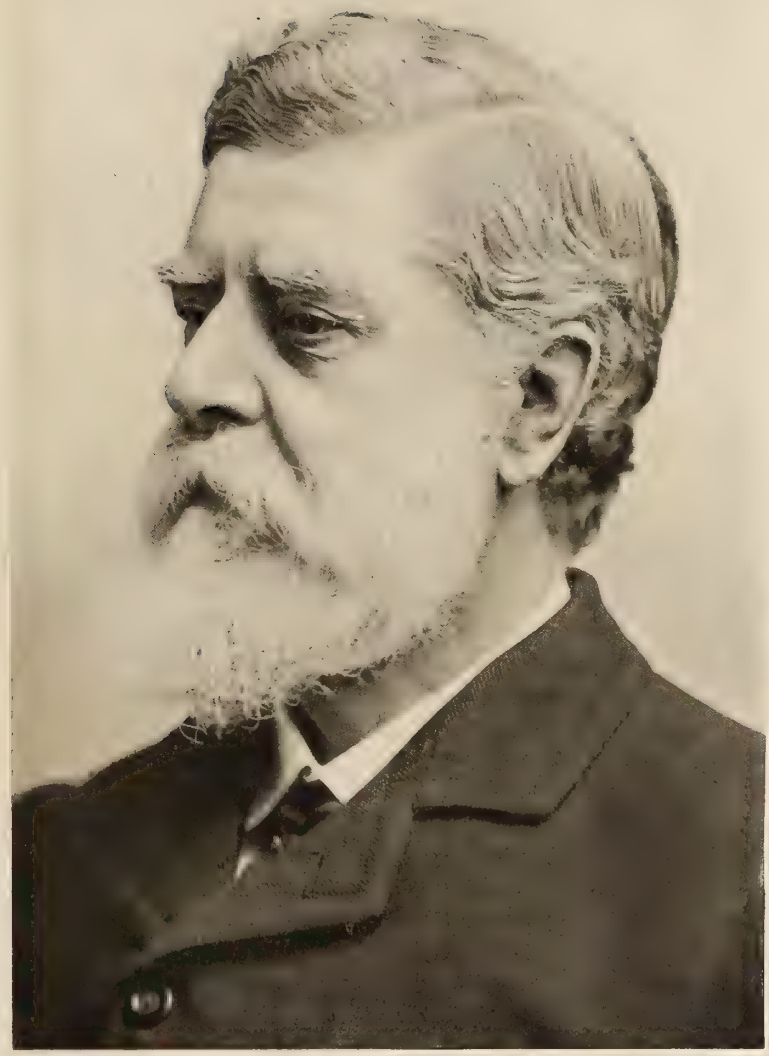
E. B. P.

ROLAND TRIMEN, 1840–1916.

Roland, the third son and fourth of the seven children of Richard and Marianne Esther Trimen, was born, October 29, 1840, at 3, Park Place Villas, Paddington. Memories of his father and of his own childhood were supplied by Roland Trimen to Sir William Thiselton-Dyer for the obituary (1) of Henry (1843–96), the youngest of the five brothers. Richard Trimen, whose "urbane address and manners of the old school" are recalled by Sir William, "traced his ancestry to a stock which, under similar names, exists both in Cornwall and Brittany." He was "a great lover of Nature, and an excellent observer; he possessed, too, a keen artistic perception, and some ability in execution. The two younger brothers were closely associated in their early bringing up. They derived from their father, both by inheritance and example, an early delight in natural objects. He continually encouraged them in their attempts to form collections of shells, insects, plants, fossils, etc., often accompanying them in country excursions, and pointing out interesting animals and plants. The elder brother remembers how, when it became necessary to restrict, in some definite direction, accumulations that were becoming unmanageable, it was solemnly decided that Henry was to occupy himself with plants, and Roland with insects." In spite of this necessary restriction of activity, Roland always retained his early love for plants and Henry for animals.

The family belonged to the "Catholic Apostolic Church" which has its Central Church in Gordon Square, W.C., Richard Trimen holding an important office in the Community. Their mother had the most remarkable knowledge of the Bible—her husband used to call her his "walking concordance"—and it is very probable that her influence was largely responsible for the admirable literary style of the two brothers. Sir Ray Lankester, who remembers her as "a very kind and charming old lady," and first met the Trimen family at Felixstow about 1859, writes:—

"Roland and Henry were great collectors of butterflies and moths and I came upon them through a butterfly-collecting friend, the Rev. Herbert Bree. They taught me to 'set' butterflies and to 'sugar,' and I enjoyed the sport, but did not keep it up. *They* were perfect adepts at it. At their home in London, in Guilford Street, Russell Square, they had a beautiful first-class cabinet and splendid collections of British species. It was at their house I first met Thiselton-Dyer who was and remained always a very intimate friend of Henry Trimen. I, too, became a great friend of Henry, who was nearer in age to me (a year or two older). Roland was regarded by us as very clever and distinctly older. Both he and Henry were full of fun and used to write comic plays and songs. Henry and I often went to theatres together and acted in private theatricals at his house and his friends' houses. I think Roland must have gone to the Cape when I went up to Oxford. I remember



Roland Trimen

writing to him to ask if he could send me *Peripatus capensis* and he did so. I don't think I saw him more than two or three times after he returned to England, though I had formed a high opinion of his value as a Darwinian worker."

Roland's health was delicate and after four or five years at King's College School, which he entered in 1853, having been previously at a private school in Rottingdean, he was advised to take the voyage to the Cape. The result was considered favourable, and he returned in 1860 to enter upon his thirty-five years in the Cape Civil Service.

On October 31, 1872, he was offered and in the following year entered upon the duties of Curator of the South African Museum in Cape Town, in succession to E. L. Layard. During his twenty-three years' tenure of this important position he was often, as he wrote to a friend in 1910, driven to despair, "during the long series of cheese-paring years which struck the chill of poverty into one's bones." Then too the time he could devote to science was greatly reduced by administrative and secretarial work, much of it outside the museum. Thus, to mention only a few of his activities, he was appointed Representative of Cape Colony at the Phylloxera Congress, meeting at Bordeaux in 1881, and, in 1892, a Member of the Committee to investigate and report on sea-fishing with a view to legislation. But it was his membership of the Phylloxera Commission from 1886, involving the inspection of vineyards in the hottest months of the year, that finally undermined his health and led to his retirement from the Curatorship and return to England in 1895. In spite of the fact that his resignation was caused by Government work quite outside his official duties, the full pension was not awarded. At the same time the reception of a pension of any kind from the Cape Government rendered him ineligible for a Civil List pension at home. Although, because of his splendid spirit and abounding humour, few would have known it, the last 21 years of his life were years of disappointment and straitened circumstances.

Roland Trimen was elected F.R.S. in 1883, his brother Henry in 1888. He was President of the South African Philosophical Society in 1883 and 1884, joined the Entomological Society of London in 1859, and was, at his death, third of the Fellows in seniority. He was President in 1897 and 1898, choosing for his two anniversary addresses the subjects of "Mimicry" and "Seasonal Variation." In 1899, during his residence at Oxford, the Hon. M.A. was conferred upon him by the University. In 1910 he was awarded the Darwin Medal of the Royal Society.

In 1883 he married Miss Henrietta Blanche Bull, daughter of H. E. Boys Bull, of Calcutta. They first met some years before in Cape Town, and not, as has been stated (5), in Paris.

Trimen, when only 16, began to record his observations on insects at Dorking, publishing in the first (1856) and later volumes of the "Entomologist's Intelligencer." The geniality and humour, so characteristic of the man, were evident in these, his earliest writings (4).

Two years after the beginning of his residence at the Cape, he began the publication of 'Rhopalocera Africæ Australis, a Catalogue of South African Butterflies' (London and Cape Town, 1862–66), expanded in 1887, with the assistance of Col. J. H. Bowker, into the classical monograph 'South African Butterflies' (3 vols., London, 1887–89). This work, although mainly, was by no means solely, descriptive; for it contains valuable introductory sections on Classification, Distribution, Sexual Differences, Habits, Protective Resemblance and Mimicry. Under each of the species, too, Trimen records the whole life-history and habits so far as they were known at the time. The literary grace which distinguishes all his writings is conspicuous in this great work, which must have cost its author immense labour. But Trimen always felt a just pride in making his descriptions clear and readable as well as precise and accurate, and, when so many might have been used, in selecting the right word for every purpose. And this, in so full and varied a language as English, must always mean immense care and pains, as well as natural gifts: must mean, in fact, the "damned hard writing" which, as an author is said to have assured an admirer of his style, is the only road to "easy reading." It is in large part a question of thoroughness, and Trimen was essentially thorough, in his work, in his correspondence, and in every relation of life.

In writing a brief account of a scientific man, and especially such a man as Roland Trimen, it is more essential to speak of what he was than of what he did. What he did may be found fully catalogued in well-known works of reference, and will always abide in the memories of those who build upon the foundations he laid down. So far as they are referred to here, his writings, being part of himself, will help to create the picture of what he was.

Roland Trimen had an impressive and most pleasing personality. "Besides being a man of strikingly handsome appearance—he might have sat for a bust of Olympian Zeus without the severity—he ever maintained a grand simplicity and gentleness of nature, combined with a charm of manner," which can never be forgotten by those who knew him (6). Although a man of great and varied gifts, he was always modest and unassuming. Examples of his verses, both grave and humorous, may be read in Mr. Bethune-Baker's excellent notice (5), but his best work is of too intimate a nature to be made public. The extent of his artistic powers would never be realised by a study of the illustrations, admirable as these are, contributed by his own pencil to his scientific works. Few, even of his intimate friends, knew of the vigour and incisive humour of the cartoons in which he caricatured the political situations at the Cape, or of his power as an actor. With his versatility and humour, and love of friendly intercourse, Trimen was the most delightful of companions. Towards the end of his life, although weakened and depressed by illness, he never failed to revive and become his old self in the presence of his friends, and, when many were present—it may have been in Common-room—to attract men with widely different tastes and interests.

As I write this and think of those pleasant times, some of the experiences he described with such humour come back to me.

On one of his visits to a European Congress he met a distinguished German astronomer, who, as soon as he realised Trimen's position at the Cape, began a fervent entreaty, with the words:—"Ach, mein frient, I am not what the world beliefs: the world knows me as an astronomer, but my heart is mit peetles!" Trimen could not resist the appeal, and the "peetles" were sent.

The Perpetual Secretary of the French Academy was expected to visit London just about the time that Trimen returned from the Cape after his election as an F.R.S. He was sitting quietly in the tea-room of the Royal Society, when one of the Officers suddenly hurried up and circled admiringly round him, with:—"Ah! Mon cher ami je suis enchanté de vous voir!" His blunt "What do you mean? I'm Trimen," was received with obvious disappointment and some annoyance.

Annoyance, too, was felt by a visitor who was terrified by the sight of a living Death's Head Moth in the Curator's Room at the South African Museum. As he kept backing away, Trimen, with the harmless insect in his hand, followed him, and, as he still refused to be reassured, could not resist the temptation to give him an experimental proof—by throwing it at him! On another occasion the sight of the same object of superstitious dread in his hand stampeded a party of fifty or sixty Dutch men and women on their way to a "Nacht-Mahl" (Lord's Supper).

On one occasion he went to see a friend in the Cape Town Post-office, and found him in the diamond strong-room. His friend was called away, and there was Trimen alone, surrounded by little parcels—a small pocketful would have meant comfort for life! It was a humorous situation, and as such made its appeal to him.

Trimen's own account of the meeting between the young German naturalist, R. von Willemoes-Suhm, of the "Challenger," and a member of the Council of the newly founded University of the Cape, has been quoted elsewhere,* but I repeat the conversation, for it is not devoid of significance even in these later years. The "Challenger" was received with the warmest hospitality, so much so, indeed, that, as Trimen told me, the epoch-making discovery of the structure and synthetic position of Peripatus was only rendered possible by Moseley's stern refusal of all invitations, in order that he might hunt for living examples of this extraordinary animal.

At a dinner given by the Governor, the Councillor remarked to the German:—"You'll be interested, perhaps, to learn that the Colony now possesses its own University."

"Ah! an University; that is good! Tell me, then, who are your Professors?"

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* 'John Viriamu Jones and other Oxford Memories.' Poulton, London, 1911, pp. 24–26.

"We have no Professors; it's an *Examining* University."

"Wait! Stop! I do not understand. How can it be? No Professors?"

"'Tis simply an *Examining* University," replied the Councillor, with some asperity.

"So! you then examine the students first, and you teach them afterwards! What you call in English 'put the cart before the horse!' Ach! but this is hellish!"

This last remark put a stop to any further talk between them during the rest of dinner. But afterwards Willemoes-Suhm said to Trimen:—

"Is not 'hellish' a good English word?"

"It is certainly not very often used in polite society, and not exactly what we should call a 'drawing-room word,'" replied Trimen.

"Ach! That is strange; for in German we say always 'höllisch' this, or 'höllisch' that; it is a quite usual word. Now I can see what made the old gentleman so angry; but what I said was true, quite true!"

In any further reference to Trimen's scientific career, I feel that his spirit will be best preserved by dwelling on the human personal side, and especially by giving his own impressions of the men he met and of their attitude towards him and his work.

He always felt that his friendship with Darwin, and the work he did as a young man under the inspiration of Darwin, were the influences to which, as a scientific man, he owed the deepest debt. It was towards the close of 1859, after his earliest visit to the Cape, that he first saw the illustrious naturalist—twice on the same day in different departments of the British Museum. The "Origin" had just come out, on November 24, and feelings were high, so much so indeed that Trimen was quite unable to get the introduction he longed for, but received instead a double warning against "the dangerous doctrines so seductively set forth by this most eminent but mistaken naturalist." "Years afterwards," he wrote, "when I had reached the honour of correspondence and personal acquaintance with Mr. Darwin, I gave him some amusement by my account of the impressive manner in which, on the first day of my seeing him, I had been warned by two naturalists, much my seniors, to give him a wide berth" (2).

The first edition of the 'Fertilisation of Orchids,' published early in 1862, at once stimulated Trimen to study these flowers at the Cape, and in less than two years he had accumulated notes and drawings which he sent to Darwin. The reply, written at Down on January 31, 1863, was the first of a long series of letters which illustrate, to use Trimen's words, "one of the most charming and attractive sides of Darwin's character—the gracious and glad welcome and recognition he never failed to extend to everyone who even in the slightest degree endeavoured to render some aid in his researches" (2). From the very first, Trimen was urged to publish on his own account, and very soon Darwin wrote:—

"I felt so sorry that such excellent work should remain locked up for an indefinite period in my portfolio, that you have made me break a solemn vow, and I have drawn up from your notes (and selected four figures for woodcuts) an account for Linnean Society. I have enlarged a little and explained and introduced a few remarks. . . . The title is 'On the Fertilisation of *Disa grandiflora*, by Roland Trimen, Esq., of the Colon. Off., C. Town: drawn up from notes and drawings sent to C. Darwin, Esq.' I hope that you will approve of this, and not object to anything in the little paper."*

The importance of this correspondence, beginning when Trimen was not much over twenty-two, and continued for nearly ten years, has already been emphasised. His whole outlook upon nature was influenced not so much by the facts as by the light in which they were approached. And yet the facts were important and varied, ranging from the Cape orchids, insect and bird visitors to various flowers, and heterostyled flowers, through many of the subjects treated in "The Descent of Man and Selection in Relation to Sex," to the geographical distribution of beetles.

The Darwin Medal was awarded to Trimen partly in consideration of his work in the establishment and illustration of the theory of Mimicry, and partly because of the assistance he had rendered to Darwin by investigating the Cape orchids. When the award was made known he wrote to a friend:—

"Nothing has given me such pleasure since my election to R.S. in 1883. . . . On October 29 I completed my 70th year, and was feeling somewhat old and depressed; but this really noble gift comes like a birthday one, and is quite rejuvenating!"

And two years later he wrote: "At this time of year I think and shall always think of the Darwin Medal award." A special resolution of congratulation was unanimously passed by the Entomological Society, and naturalists generally would agree with the opinion expressed by Sir William Thiselton-Dyer, in a letter to the present writer, that the award was "essentially typical of the original idea, *i.e.*, thoroughly Darwinian, and such as would have greatly pleased Darwin himself."

The world has now lost the last of the six great naturalists who laid the foundations of the study of insect Mimicry—Charles Darwin, H. W. Bates, Fritz Müller, A. R. Wallace, Raphael Meldola, Roland Trimen.

It was in this subject that Trimen's greatest scientific discovery was made—the unravelling of the tangled relationships of *Papilio dardanus* or *merope*, as it was then called, and the interpretation of the widely different female forms on the theory of Mimicry. The discovery, indirectly due to Darwin, directly to Bates and Wallace, was at first received with indignation and ridicule, but Trimen lived to see all the forms he disentangled, as well as others then unknown, bred from the eggs laid by a single female, and to see this repeated in far distant localities where the forms differed in correspondence with their local models.

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* 'Journ. Proc. Linn. Soc. Bot.,' vol. 7, p. 144 (1863). This and Trimen's further investigations on the Cape orchids are fully recognised in the second edition (1877) of Darwin's book.

A few years before his death Trimen was persuaded to write the following hitherto unpublished memories of his investigations and the impatience with which they were received by a naturalist of the old school:—

"Towards the end of 1867 I was in London, working at a paper* which I had in preparation 'On Mimetic Analogies among African Butterflies.' Earlier in the same year I had enjoyed some delightful months of collecting and observing in Natal, and it was some of the material thus obtained that I was comparing with the tropical African forms in the British Museum, the Oxford University Museum, and various private collections. I was therefore only too glad to accept a very kind invitation from Mr. W. C. Hewitson to stay a few days with him at Oatlands, and inspect his celebrated collection of the butterflies of the world.

"Most hospitably was I entertained, and unlimited access to the cabinets was accorded me. My knowledge of the South African species, after eight years' collecting and study of them, was of service to my host in the determination of various little known or closely-allied forms—and this he recognised and welcomed.

"But my mind was full of the mimetic relations of butterflies, and of my quite recent acquaintance with them in life; I was ardent and enthusiastic on this fascinating theme; and although I had gathered from Mr. Hewitson's publications—and, indeed, had been told by several entomologists—that he took little more than a 'mere collector's' view of the insects which he amassed and illustrated so assiduously, I could not refrain from every now and then pointing out some of the more striking instances as I came across them in his cabinet drawers. Though Mr. Hewitson repeatedly scouted and even ridiculed the idea of mimicry, he was not, as far as I can remember, really much moved until I came to the three all different Danaine-imitating females of the southern form of *Pap. dardanus*, then known as *P. merope*. What seemed actually to disturb him most was my venturing to suggest that, although the species-identity of all four had not been absolutely proved, this was so sure a case that he would be quite safe in at once placing the three females *cenea*, *trophonius*, and *hippocoon*—then widely separated from their proper mate by a good many drawers full of other Papilios—with *P. merope*. This was too much for his equanimity; and he very decidedly expressed his opinion that my notion was altogether fantastic and nonsensical.

"I was anxious to avoid occasioning any irritation to one so much my senior, and so truly devoted to artistic illustration of the Rhopalocera, and accordingly said no more just at the time. But, before leaving, I took an opportunity of remarking how often the accurate mimicry of a Danaine or Acræine by species belonging to entirely distinct groups led to the erroneous location in collections of a mimicker with the family or genus, or even sometimes the actual species of the mimicked form. He was not at all disposed to admit this; so I told him that I had noted a case in his own collection—not of such great exactness as those above mentioned, but so near in the common likeness that what was unquestionably a Lycænid had been placed among the Pierinæ and labelled "*Pieris*" *erastus.*[*] He showed the greatest annoyance at this, declaring that I was absolutely mistaken, nor would he allow me to point out to him the Lycænid structure of *erastus.* When I a little pressed him to examine the *feet* of this butterfly, he rose from his drawing-table, came across to the cabinet I was inspecting, and quite indignantly put an end to the discussion by pushing the drawer back into its place!

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* Read March 5, 1868, published in 1869 in Part III of 'Trans. Linn. Soc.,' vol. 26, p. 497. The volume was not completed till the following year, and is therefore dated 1870.

"All the same, we had quite a friendly parting; and, six years later, in a part of his 'Exotic Butterflies,' published in October, 1873, he quietly removed to the Lycænid genus *Liptena* the erst misplaced '*Pieris*' *erastus,* later on referred by Kirby to his genus *Citrinophila.*—R. T., 28–vi–1912."

Trimen's youth lay in days when, as I have been told, one met the scent of the Hampstead hayfields in Gower Street, and it seems fitting that, in such a setting, in an atmosphere so remote, he once saw, and ever afterwards preserved the memory of, that aloof, secretive figure, William John Burchell, greatest of African travellers and writers. And so, about fifty years later, he drew for me one of the very few pictures of that great man:—

After dwelling in admiration upon the "singular distinction" of Burchell's 'Travels in the Interior of South Africa,' its "comprehensive and many-sided record of faithful observation in every branch of natural history," its "rare qualities of sagacity, insight, and width of grasp," and its "style of sustained dignity and lucidity," Trimen continued:—

"When I first visited South Africa, in 1858, I was not a stranger to this notable book, having consulted it before leaving England; and during my long residence at the Cape I found it of the greatest value. My dear friend, the late Mr. Charles A. Fairbridge—a founder and trustee of the South African Museum, possessor of the finest private library in South Africa, and of a wide and critical knowledge of English literature—was an absolute enthusiast as to the merits of Burchell, whom he pronounced to stand alone in the combination of scientific and literary merit.

"I was so fortunate as to have seen Burchell on one occasion. It was in the Insect Room of the Zoological Department of the British Museum, where (towards the close of the year 1857) I was engaged in noting what Lepidoptera were then known to inhabit South Africa, so as to have some guide respecting the species I might look out for on my approaching visit to that country. The Insect Room was then in charge of Mr. Adam White, most amiable and obliging of curators, and I was, as usual, consulting him on all sorts of points when an old, rather stooping, white-haired man came quietly into the room and asked the attendant if he could see Mr. White. As the visitor approached and entered the fuller light from the window near which I was sitting, I was struck with the character in his face, which, still handsome, was marked with dignity and refinement, but was singularly cold and hard in expression. White rose to receive him, but the stranger did not return his smile, and seemed to say 'Good morning' as a necessary formality only, proceeding to ask White to show him some group of insects. The two then went to a distant cabinet, where the visitor made some notes, but very soon left. White came back to his seat and presently asked me if I knew who it was that had called, adding immediately that it was none other than Mr. Burchell, the great South African traveller and naturalist.

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[*] The female is a good mimic of a common type of pattern in the African Pierine genus *Mylothris.* The under surface of the wings of both model and mimic is white or creamy, with a marginal black beading, and an orange flush at the base—just where it would appear in the resting position.

"'Ah, sir,' he went on (this was his usual way of beginning a sentence), 'a great man indeed, with many gifts and of great learning, but terribly *soured.* I fear he'll never get better of it now. 'Tis a pity indeed to have that proud and unforgiving spirit,' and so forth. White then, with bated breath and intimation that what he said was confidential, told me that Burchell had for long cherished a bitter grievance against the Trustees of the Museum, who had (according to his view) done him irreparable wrong by their neglect of certain of his zoological collections which had been taken into their charge, and so allowing the specimens to be destroyed by insects and damp. I was quite a youth at the time, and this moving tale made a great impression on my mind, and invested Burchell's personality with a certain grimness which I could not forget."

After his return from the Cape in 1895 Trimen experimented with many places in the London district in the hope of finding a neighbourhood that would suit his health. Twice he lived at Oxford—the second time for three years (1907–1910). He felt the strain and expense of the numerous removals and especially the anxiety of taking with him his collection of African butterflies, now in the possession of Mr. J. J. Joicey at the Hill Museum, Witley.

In the early summer of 1916 his health was evidently failing, and on July 4 he entered Dr. Chipperfield's nursing home at Epsom, where he gradually sank and died on July 25. I saw him for the last time on June 30. He had fallen down a few weeks earlier, having tripped over a grating, and looked very ill and depressed; but he was cheered, as he wrote in his diary, at the sight of an old friend and by hearing of an interesting new observation* on his old favourite *Papilio dardanus.* It had just been discovered that males from the island of Fernando Po differ in certain respects from those on the opposite West African Coast, but resemble the males of the East Coast and of Madagascar. The name of the butterfly awoke his interest directly, but, as soon as he heard of the island, his face lighted up in a flash. I knew well what was in his mind. His thoughts had gone back to Cape Town and to a late sitting of Parliament, where a member quoted some lines from, so he said, Edgar Allan Poe.

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* 'Proc. Ent. Soc. Lond.,' 1916, p. xciii.

"I am sorry to detain the House," said John X. Merriman, "but really I know Poe's works pretty well and I feel confident that the lines quoted by the Hon. Member are not to be found in them."

"I apologise to the House," was the reply, "the lines were not by Edgar Allan Poe, but by his brother Fernando Po!"

The scattered impressions and incidents here recalled are for the most part gathered from my memories of a dear friend, but much is owing to the writers of the following obituary notices, to Trimen's elder brother Mr. Edward Trimen, and, above all, to his wife.

On summer days the butterflies he loved so well, attracted by the purple flowers of a Buddleia, hover over his grave on the heights of Highgate, and all who knew Roland Trimen will feel the fitness of the inscription under his name on the plain grey stone—"The Well-Beloved."

1. "Henry Trimen, 1843–1896," 'Proc. Roy. Soc.,' vol. 75, p. 161, 1905, by Sir William T. Thiselton-Dyer.
2. 'Charles Darwin and the Origin of Species,' London, 1909, by E. B. Poulton. 'Letters from Charles Darwin to Roland Trimen,' in, pp. 213–246. See also p. 28, n. 2.
3. 'Nature,' vol. 97, August 10, 1916, p. 485, by E. B. Poulton.
4. 'Entomologist's Monthly Magazine,' vol. 52, September, 1916, p. 209, by J. J. Walker.
5. 'Entomologist's Record,' vol. 28, October 15, 1916, p. 231, by G. T. Bethune-Baker.
6. 'Entomologist,' vol. 49, October, 1916, p. 240, by H. Rowland-Brown.
7. 'Proceedings of the Linnean Society of London,' 129th Session, October, 1917, p. 76, by E. B. Poulton.

E. B. P.

THOMAS GREGOR BRODIE, 1866–1916.

The death of Prof. Brodie occurred on Sunday, August 20, 1916. His sudden end was totally unexpected by his friends. He was subject to attacks of gout, and he experienced one which was not of a severe character during the week preceding his death, but sitting up in bed on the Sunday morning and feeling better, he was seized with heart failure and expired within a few minutes.

He was the second son of the late Alexander Brodie, Vicar of Grandborough, and was born at Northampton in 1866, so when he died he was just over 50 years of age, in the prime of life, and at the zenith of his intellectual power. The tragedy is intensified, for the victim was one of the most original and gifted of our company of Physiologists.

He received his education at King's College School, whence he proceeded to St. John's College, Cambridge, but after being there a year or so he decided to make medicine his career, and chose King's College, London, as his school. There and at King's College Hospital he was one of a band of exceptionally brilliant students, several of whom are now on the staff of that hospital, and among them Brodie was the leading spirit in both work and play. He carried off the principal prizes the College had to offer, and would have proved eminently successful in any branch of medicine he had chosen to take up. His choice, however, fell on Physiology, and after he had taken his M.D. at the University of London, he was appointed Principal Assistant to the Professor of that subject in King's College in 1890. He subsequently became Senior Demonstrator at the London Hospital, a post which he soon relinquished for the Lectureship in Physiology at St. Thomas's Hospital to which he was elected when Dr. Sherrington became Professor at Liverpool. A few years after this he received the Directorship of the Laboratories of the Royal College of Physicians and Surgeons on the Embankment. He, therefore, gave up active teaching and his new post was one which specially suited his capabilities and temperament. Whilst there he gathered round him a band of workers whom he inspired with his energy and contagious enthusiasm, and the research work issuing from his laboratory was not only large in quantity but of the highest quality and value. A few years later a fit of economy seized the Royal Colleges, and they decided to give up this valuable but expensive branch of activity, so that Brodie for a time was without a post. This did not last long, for within a few months he received, almost simultaneously three new appointments, viz.:—The Professorship of Physiology at the Royal Veterinary College, the Lectureship on the same subject at the London School of Medicine for Women, and the office of Professor-Superintendent of the Brown Institution under the University of London. This pluralism was necessary, for all posts were badly paid, and even a combination of any two would have been insufficient to provide a living wage.

This is an illustration of the way scientific workers are rewarded in this country. But his determination and energy carried him through; he performed his duties with success in all, and original researches continued in spite of his other duties to issue from his laboratories, which had been carried out by himself and his colleagues. But though his energy was untiring, it was a dog's life that he led, and it was his undaunted spirit alone which overcame his physical fatigue. In 1904 he received his F.R.S., and until 1908 he continued in the unsatisfactory position of successfully serving three masters. In the last-named year, he received an offer of the Chair of Physiology in Toronto University, its previous occupant, Prof. Macallum, having decided to devote his work to Biochemistry. In the end Brodie accepted the invitation and held this Professorship until his death. For domestic reasons the most important of which was the education of his sons, he continued to keep on his London house, and here (in Hampstead) he spent the long vacations which Canadian Professors enjoy. Not that he took much real holiday, for research work continued to occupy his time. The Toronto Professorship was no sinecure; in addition to his heavy teaching responsibilities, he had to organise his laboratory, and he made the bare bones of the new buildings placed at his disposal live, and his laboratory blossomed into one of the most complete in the world. It was replete with every modern convenience and the generosity of the University and of the Canadian Government enabled him to make its equipment second to none. For the first time in his life he was freed from niggling considerations of sparing expense, and his famous laboratory still lives as a monument to his wise use of public money.

In 1915, he came over here in a different capacity, namely, as a Captain in the Canadian Medical Service, and here his ability for original thought and work proved invaluable. In this country his work was carried out at the London School of Medicine for Women, at the Endell Street Hospital, and later at the Military Hospital, Ramsgate, where also he worked in the summer of 1916, the date of his death. Respiratory changes in disease and injury formed his *magnum opus* in this connection, also the means of re-educating maimed men to enable them to resume a useful life.

His published papers make a long list, and their subjects embrace nearly every branch of physiological science. But his main bent, as was foreshadowed by his first paper on the 'Elasticity of Muscle,' was physical. He will, perhaps, be best remembered for his epoch-marking work on the Kidney, and his views on the function of the Glomeruli formed the subject of his Croonian Lecture delivered before the Royal Society. But whatever his subject, his great originality was his most marked characteristic, and his never-failing resourcefulness in overcoming difficulties by the use of new methods and apparatus made his name famous. He published also a 'Textbook of Experimental Physiology,' which still remains a standard and a pattern to his successors in the book-making line. He was not a ready writer, but all he wrote was a model of care and thoughtfulness. He made no pretension of being an orator, but again all he said was the result of thoughtful carefulness. He succeeded in arousing the interest of his listeners, and the respect and affection of his students and colleagues. His incisive manner compelled attention to his lucid expositions.

As a friend, words fail to do him justice. He was a friend worth having, loyal, affectionate, bright, and delightful in every sense.

Outside of his profession and professional work he had many interests. He was a keen student of literature and collector of books. In all sports, but especially golf, he was as keen as a boy. He was never happier than when spending a day bicycling with his friends—a leader in all their fun and gaiety, or in teaching his sons the rudiments of carpentering. He had been married 22 years; his widow and three fine sons live to mourn his loss.

His funeral took place at Hampstead on August 23, 1916, and this last sad ceremony was carried out with full military honours. It was attended by representatives of the Canadian Army, of the University of Toronto, of the various institutions in London with which he was or had been connected, of the Royal Society, and other public bodies. Among those who gathered round the graveside were a host of personal relations and friends, many of whom hurried back from their summer holiday to pay him this last tribute of respect. The scene was a memorable one, and there were but few dry eyes when the bugles sounded "The Last Post."

Owing to misunderstandings, the publication of this obituary notice has been long delayed. But even after a lapse of nearly four years, the sense of personal loss is as great as ever; whilst in the scientific world, the gap has never been quite filled up. The circumstances of his strenuous life of struggle betrays that indifference to the work of science which our country has been ever wont to exhibit. The recent war has brought home to the nation a lesson which ought to be taken to heart, and not forgotten now that peace, which has its troubles too, is once more with us. These few lines tell briefly the story of a man with exceptional powers and brilliant gifts who was unable, nevertheless, to obtain in his native land a position and a competence worthy of his greatness.

W. D. H.

HORACE BOLINGBROKE WOODWARD, 1848—1914.

THE subject of this notice was the third of a generation of geologists, his father being Samuel P. Woodward, the well-known naturalist and palæontologist of the British Museum, and his grandfather Samuel Woodward, one of the early writers on the geology of Norfolk, to the knowledge of which the grandson was destined to add so much.

Born in London on August 20, 1848, Woodward was educated at a private school; but his geologic education was largely evolved, as in the case of many other men, during his work on the Geological Survey. He married Miss Alice Jennings in 1873, and lost her in 1902. They have left but one daughter.

The last few years of his life were marred by serious illness, but this did not prevent him from pretty constant activity in the work of writing; indeed his industry was marvellous. He bore his long illness with fortitude, maintaining a cheerful spirit to the last. He died at Croydon on February 6, 1914.

Woodward spent the greater part of his life on the Geological Survey, on which he served for 41 years, adding greatly to its reputation, and gaining the esteem and affection of his colleagues. He was appointed Assistant Geologist in July, 1867, promoted to Geologist in January, 1875, and became Resident Geologist, in London, in December, 1894. He was promoted to District Surveyor two years later, was made Acting Director for England and Wales in July, 1899, and had his final promotion to Assistant Director in April, 1901, which post he held until his retirement on the last day of 1908.

His field-work on the Survey was of a varied kind, chiefly amongst formations of Pleistocene, Pliocene and Jurassic age. He spent much time in Somerset (including the coal-field) and neighbouring counties. He took part in the Drift-mapping of the country around London; he had a long experience of Norfolk (the county of his grandfather, Samuel Woodward); and he had to examine the many counties of central and southern England in which Jurassic beds occur (for the preparation of his great Memoir on those beds), besides which he also did work on rocks of that age in Scotland. As Assistant Director he had of course to deal with all the formations of England and Wales, in superintending the work of the staff.

His connection with the Geological Society was a long one. He was appointed Assistant in 1863, and was elected a Fellow in 1868. He served on the Council for several years, and was a Vice-President in 1904–6; but bad health in later years prevented him from taking part in the affairs of the Society. The Council awarded him the Murchison Fund in 1885, the Murchison Medal in 1897, and the Wollaston Medal (its highest honour) in 1909. He was President of the Geologists' Association for a year (1893–4); but his official duties hindered him from continuing to hold that office into the usual second year. During his stay in Norfolk he was President of the Norwich Geological Society and of the Norfolk Naturalists' Society, as well as of the local Science Gossip Club. In 1896 he was elected into the Royal Society.

Of Woodward's many works one may select three books, of different kinds, as sure to be an enduring monument of his geologic ability in various ways. The first is an official memoir, vols. iii–v, of 'The Jurassic Rocks of Britain,' published in 1893–5, which gives a detailed description of a great group of geologic formations, based on his own careful examination, coupled with an exhaustive knowledge of the works of other observers. The second was written for one of our learned societies. 'The History of the Geological Society of London,' published in 1907, is a fine addition to the literature and history of geology, as evidenced by the progress of the senior geologic society of the world.

The third was a private venture, namely, 'The Geology of England and Wales,' first published as a comparatively small book. The second and greatly enlarged edition (practically a new book), issued in 1887 and long out of print, shows a marvellous acquaintance with the work of geologists in the country with which it is concerned, as well as great power in codifying, so to speak, the many branches of that work, so as to give a general view of all the rocks of the kingdom, with fitting details, and with references to the sources of original work. This book is greatly valued not only by geologists but also by engineers or others who are concerned with the practical application of geology.

These three books show great industry, widespread knowledge, and the gift of doing exactly what was wanted. The Geological Survey was fully justified in entrusting one of its most important memoirs to Woodward. The Council of the Geological Society was also justified in getting him to undertake the arduous task of writing the history of the Society. Woodward himself was justified in undertaking the difficult problem of producing a book that would epitomize the geologic record of England and Wales.

Besides these larger books he was also the author of several others: 'Memorial of John Gunn,' which is a contribution to the geology of Norfolk (1891); 'The Geology of Water-Supply' (1910); 'The Geology of Soils and Substrata' (1912); and a short 'History of Geology' (1911).

He was also author or part author of many geological survey memoirs on various districts, notably on the East Somerset and Bristol coal-fields (1876), and on the country around Norwich (1881); and of course also of many sheets of the map.

The later editions of Stanford's 'Geological Atlas of Great Britain and Ireland,' making it an indispensable work to students of the geology of these islands, was edited by him, and he was also an assistant editor of the 'Geological Magazine.' Finally he contributed many papers, addresses, etc., to many scientific journals, and his name is prominent amongst those who have done much for the geology of his native land. W. W.

JAMES GEIKIE, 1839—1915.

James Geikie was born in Edinburgh in 1839, and educated partly at a private school, partly at the High School and the University of his native city. After leaving school he served an apprenticeship for several years to Mr. Constable, the printer, but the strain and drudgery of the work compelled him eventually to terminate the engagement. From his early days he showed his liking for nature study during his leisure hours and holiday rambles in butterfly hunting, collecting fossils, examining drift sections, and in trying to interpret the records of the rocks, so well displayed in the crags and ravines in the neighbourhood of Edinburgh.

He mind was clearly bent in following natural science as a career in life, and, in 1861, he fortunately obtained an appointment on the staff of the Geological Survey. His colleagues at that time in Scotland were H. H. Howell, Archibald Geikie, John Young, and B. N. Peach. During his service of twenty-one years he mapped large areas of the Scottish coal-fields, portions of the Older Palæozoic formations of the Southern Uplands and the Cheviots, together with tracts of Old Red Sandstone and the metamorphic rocks of the Highlands in the counties of Perth and Forfar. His work in the field gave him a thorough grasp of the structure of the Scottish coal-fields, which naturally led him to take a keen interest in tectonics.

The most striking feature in his scientific career is the influence which he exercised on the development of glacial geology. His researches in this department stimulated enquiry and aroused keen opposition. He was recognised as one of the foremost leaders of a distinct school of glacial geology.

When James Geikie began to map the superficial deposits in 1861, the early views of Lyell, Darwin, de la Beche, and Murchison, that the transported blocks, stony clays, sands and gravels had been the work of floating ice, were widely accepted, although Agassiz, as a result of his visit to this country in 1840, had demonstrated that they were due to the action of land ice and glaciers. The land ice theory had been adopted and confirmed by Buckland, Robert Chambers, Andrew Ramsay, Archibald Geikie, and T. F. Jamieson.

The rival theories led James Geikie to concentrate his attention on working out the history of these deposits. It was a matter of great gratification to him that he spent the first two years of his official life in surveying the boulder clays, sands, gravels and peat in those areas where only the solid geology had been shown on the maps. It gave him excellent opportunities of studying the distribution, the characteristic features, and the relation of these materials to the solid rocks underneath. As the work proceeded, he evolved certain ideas regarding oscillations of climate in Pleistocene time, based on the succession of boulder clays, with intercalations of sand, gravel, and peat, and on the Cave deposits and Palæolithic gravels in the south of England.

In 1867, James Geikie's views were strengthened by his contact with James Croll, who had been appointed Secretary to the Scottish Staff of the Geological Survey under the Directorship of Sir Archibald Geikie. Croll's memoir on the 'Physical Causes of Changes of Climate during the Glacial Epoch' had attracted the notice of geologists, astronomers, and physicists. Accepting his theory, it followed that the Glacial Epoch must have consisted of a succession of cold and warm periods, the warm periods of one hemisphere corresponding with the cold periods of the other. The field evidence, as interpreted by James Geikie, seemed to harmonise with the results of Croll's speculative researches. The writer of this notice has a vivid recollection of the keen discussions bearing upon glacial questions which took place in the Edinburgh Survey Office for several years after 1867. Croll's commanding personality had a considerable influence on the development of James Geikie's views.

An outline of James Geikie's researches first appeared in a series of articles in the 'Geological Magazine,' but the author lost no time in expanding those articles and presenting the evidence in his volume 'The Great Ice Age,' published in 1874. This volume at once arrested the attention of geologists all over the globe. For the first time it gave a systematic account of the Glacial Epoch, with special reference to its changes of climate. It furnished a detailed description of the glacial and post-glacial deposits in Scotland, which was followed by a summary of the evidence then available regarding the relics of post-Tertiary time in England, Ireland, Scandinavia, Switzerland, and North America. Special emphasis was laid on the palæontological evidence indicating interglacial mild conditions amongst the oldest glacial deposits. The peculiar assemblage of northern and southern forms in the Palæolithic gravels and Cave deposits in England was attributed to oscillations of climate and not to seasonal migrations. With regard to the age of the Palæolithic deposits it was maintained that they were either pre-glacial or inter-glacial, and not post-glacial.

The second edition of 'The Great Ice Age,' which appeared in 1877, the volume on 'Prehistoric Europe' (1880), the third edition of 'The Great Ice Age (1894), and the Munro Lectures on 'The Antiquity of Man in Europe' (1914), mark successive stages in the evolution of James Geikie's views. The distinctive feature of the last of these volumes was the correlation of his series of inter-glacial periods with the culture stages of Palæolithic and Neolithic man. His great aim was to keep himself abreast of the increasing volume of research in glacial geology, the results of which were communicated to him by investigators from far and near. From time to time he modified his opinions regarding the interpretation of particular details in the history of the period. But the fundamental points of his teaching, that the Ice Age was characterised by a succession of cold and genial periods, and that man then lived in Europe, were never abandoned by him.

The views advanced by James Geikie regarding climatic changes in Pleistocene time have been adopted by many investigators in Europe and America. Reference might be made to the elaborate investigations of Penck and Brückner indicating four glaciations of the Alps, separated by inter-glacial periods. His classification of glacial and inter-glacial periods has been subjected to severe criticism. Great diversity of opinion still exists regarding the interpretation of many glacial phenomena, the correlation of deposits in widely separated regions, and the sequence of conditions. But sufficient palæontological evidence has been obtained to establish the general principle of oscillations of climate in the Glacial Epoch, though the number of inter-glacial periods may remain a subject of keen controversy.

In 1882, on the promotion of his brother, Sir Archibald Geikie, to the post of Director-General of the Geological Surveys of the United Kingdom, he was appointed to the Chair of Geology and Mineralogy in the University of Edinburgh. During his tenure of the Professorship he succeeded in establishing a lectureship in Petrology, a lectureship in Palæontology, and a museum collection for teaching purposes. By the encouragement of research in his laboratory and in the field, he sent forth students who have made important contributions to geology and who hold prominent positions at home and abroad. As an administrator within the University he was equally successful, for when the Science Faculty was established in 1894 he was appointed the first Dean—a position which he held for nineteen years.

In 1884 he took a prominent part in the foundation of the Royal Scottish Geographical Society, which has achieved remarkable success. He was one of the first vice-presidents, and he held this office till his death, except during his occupancy of the presidential chair, from 1904 to 1910. In 1888 he became honorary editor of the Society's magazine, and contributed articles to its pages. On his retirement from the presidentship he was presented with his portrait, in recognition of his devoted service.

His power as a clear expounder is shown in the production of other volumes in addition to those already mentioned. In 1898 he issued a volume on 'Earth Sculpture, or the Origin of Land Forms'; in 1913, a work on 'Mountains, their Origin, Growth, and Decay'; in 1898, a text-book on 'Structural and Field Geology,' which passed through three editions.

Among the honours which fell to him in recognition of his scientific researches, the following may be mentioned: the Fellowship of the Royal Society, 1875; the Presidentship of the Royal Society, Edinburgh, in succession to Sir William Turner, 1913; the Mackdougall-Brisbane Medal, awarded by the Royal Society, Edinburgh; the Murchison Medal, by the Geological Society; the Gold Medal, by the Royal Scottish Geographical Society.

J. H.

GUSTAF MAGNUS RETZIUS, 1842–1919.

Gustaf Retzius, who died on July 21, 1919, was the son of Anders Retzius, well known as an anatomist and anthropologist. He was born October 17, 1842. He first became known as a histologist, from the publication of a joint monograph, written in conjunction with Axel Key, on the structure and arrangement of the membranes of the brain and spinal cord. Somewhat later, viz., in 1872, at the age of 30, he issued the first instalment of a series of important works on the auditory organ of Vertebrata, "Das Gehörlabyrinth der Knochenfische." Both this and those which followed contain a wealth of detail regarding the minute structure of the organ of hearing in all classes of Vertebrata, and include a much more precise description of the human cochlea than had hitherto been furnished. They were published in folio form under the title "Das Gehörorgan der Wirbelthiere." It may be confidently stated that, if the fame of Gustaf Retzius rested on his works on the auditory organ alone, it would have been established upon an enduring basis.

He was, however, in no way inclined to rest upon the laurels thus earned, and was fortunate to find in the newly-described methylene-blue method of Ehrlich, and chromate of silver method of Golgi, invaluable means wherewith to pursue investigations upon the nervous system, which from now on largely occupied his attention. In this pursuit he by no means restricted himself to vertebrates, but employed, for the elucidation of his subject, material derived from every description of animal—especially annelids, molluscs, and crustaceans. He lost no opportunity which presented itself of obtaining material for his work; the writer of this notice well remembers meeting him one morning in Rome on the Palatine Hill, combining archæological studies with a search for scorpions!

The numerous and important histological observations which Retzius found time to make after the publication of his monographs on the organ of hearing were for the most part issued to the world, not by the ordinary channel of journals or transactions of learned societies, but collected in separate folio volumes, replete with illustrations; these were published annually during many years under the title "Biologische Untersuchungen." This procedure was only possible owing to the fact that he had ample private means, a circumstance which also enabled him to present the successive volumes, as they appeared, to his friends and colleagues. The Nervous System of Crustacea was treated of in a separate monograph (1890). His studies in comparative histology enabled him clearly to demonstrate the mode of evolution of sensory nerves—a problem which intimately concerns the whole physiology of the nervous system. Amongst other histological subjects dealt with in his works, not the least important is that of the structure of the cell, and especially that of the generative cell.

Gustaf Retzius

The eminence of Retzius as a histologist was duly recognised in his own country by the foundation for him in 1877 of a special Professorship of Histology at the Karolinska Institute in Stockholm. He did not, however, hold this many years, but resigned the position in 1890, in order that he might be free to carry on his researches without having to consider the claims of professorial duties.

But it was not merely as a histologist that he became distinguished. He was the founder (in 1873) and first Secretary of the Anthropological Society of Stockholm, and the author of many important works on anthropology, of which those on Finnish Crania (1898), and on Ancient Swedish Crania (1899), were alone sufficient to establish his reputation as one of the first authorities on the subject. "Swedish Anthropology" (1902) was written in collaboration with Fürst.

As an anatomist, he devoted special attention to Cerebral Topography, as is evidenced by the valuable monographs on the Human Brain and on the Brain of the Apes, which were issued in 1892 and 1906 respectively. In one of the latest publications from his pen (1914), he deals with the question whether the human brain has undergone any increase in size as a result of the progressive development of intellectual culture.

Retzius' activities were not confined to science. He had great literary ability. This is apparent in the clear manner in which he describes his scientific investigations, and is strikingly manifested in the biographies of eminent men which came from his hand—amongst them Linnæus, Berzelius, Pasteur, Scheele, Huxley, Virchow. He travelled extensively both in the Old and New World; his interests were universal, but the ethnology of the countries he visited had perhaps most fascination for him. His account of Egypt in "Pictures from the Land of the Nile" (1891) is a treasure-house of brilliant descriptive writing, and the same may be said of his "Pictures of Sicily," which followed closely upon it. He was a poet of no mean order, and possessed considerable artistic talent, a qualification which not only added to the interest of his travels, but was of utility in the illustration of his scientific observations.

Retzius was a man of unusual charm. He had many friends in all countries; not a few in Great Britain, where he was always a welcome visitor; they included Charles Darwin and Thomas Henry Huxley.

Elected a Member of the Swedish Academy in 1902, and a Foreign Member of the Royal Society in 1907, he was in 1908 invited to deliver the Croonian Lecture—the subject selected being "The Minute Structure of the Nervous System." In the following year he gave the Huxley Lecture before the Royal Anthropological Institute, on "The so-called North European Race of Mankind."

He married Anna de Hierta, daughter of Lars Johan de Hierta, founder of the well-known newspaper 'Aftonbladet.' The union was a happy one. Accompanying him in his travels, and interesting herself keenly in his work, his wife has been a true helpmeet to him, whilst he, on the other hand, has played an active part in furthering her schemes for the amelioration of the social conditions of their less fortunate fellow-countrymen. Together they have long exercised a gracious hospitality, which has caused their house to be a pleasant place of reunion, not only for persons eminent in literature, science, or politics, but also for younger men, who had yet to make good their position, and were often aided to do so by the encouragement they there received. Few will be more missed than Gustaf Retzius. But, although his name is now only a memory, it is the memory of a genial personality, of an accurate scientific observer, and of a valued friend.

E. A. S. S.

LUDIMAR HERMANN, 1838–1914.

Ludimar Hermann was born in Berlin in 1838. He appears to have learned to read at the age of three years and showed inclinations towards a scientific career at an early age. He attended courses in Natural Science and Medicine at the University of his native town, and after some interruptions by duties as medical officer in the wars of 1859 and 1864, he became assistant to Du Bois Reymond. Owing to the position Hermann took up with regard to the nature of the electrical phenomena in muscle, to be referred to presently, his relations with the professor became somewhat strained, and in 1868 he was elected to the Chair of Physiology in the University of Zürich. He remained in this position until 1884, when he was called to Königsberg. A busy life of investigation and scientific activities of a more public nature occupied his time to the full until the year 1909, in which he was attacked by an intestinal growth, which necessitated several operations, by which cure was apparently effected. He retired from his chair; however, four years later and a year afterwards the disorder returned, developed rapidly and resulted in his death on June 5, 1914, when he had reached the age of nearly 76 years.

It was Hermann's fortune to be concerned with the investigation of certain problems in physiology which were much discussed at the time. Du Bois Reymond had propounded the view that the electrical currents to be obtained from muscle when electrodes are placed, one on a natural longitudinal surface, the other on a cross-section, are to be accounted for by the presence of a series of "electromotive molecules," positive in the middle, negative at both ends, arranged in regular order. The existence of such permanent structures was disproved by Hermann when he showed by careful experiments that an uninjured muscle is equipotential over the whole surface, and that a difference of potential only arises when the cells under one of the electrodes are injured. This was a fundamental discovery and, although at that time the source of electromotive force in changes of distribution of ions and the properties of semi-permeable membranes was unknown, the facts brought to light by Hermann were a necessary preliminary to further work and led him to formulate his well-known "*alteration-theory.*" According to this theory, any spot whose chemical nature changes in the direction of injury or death becomes electro-negative in the sense of the zinc pole of the Daniell battery. We may note that stress is here laid on chemical processes, which are known to occur, in contradistinction to the purely hypothetical view of Du Bois Reymond. When we remember that this work was done in the laboratory of the latter, we realise that some courage was manifested in publishing it.

A further step was taken by showing that any part of a muscle in a *state of activity* is also electro-negative to a part at rest. Thus, there is a similarity in the nature of the process of contraction to that which is associated with injury, as already known in connection with the production of acid in activity and in *rigor mortis.* Accurate and delicate apparatus was devised in order to follow the time course of the "current of action," which was found to progress in the form of a wave from the point stimulated. If the electrodes were situated at unequal distances from such a point, that one nearest to it became negative first, the further point following with a similar change. By this means, the galvanometer showed a deflection first in one direction, then in the opposite direction, the so-called "*diphasic variation.*" Any two points in the same stage of activity are equipotential. An important practical application of these facts will be mentioned later.

The chemical or rather physico-chemical point of view taken in the preceding researches naturally led on to further investigation of the *nature of muscular contraction.* It was first shown that muscle is capable of executing a prolonged series of contractions *in vacuo.* Hence the energy is not derived directly from an oxidation process, but from some store of potential energy present in the resting muscle. Hermann found that carbon dioxide was given off, although it was reserved for Fletcher at a later date to show that this was derived from a secondary reaction not involved in the essential process.

Hermann called the hypothetical substance from which the contractile energy was obtained, *inogen,* and in the belief that carbon dioxide was evolved in its decomposition, inogen was interpreted as a compound of a protein constituent of muscle ("myosin"), carbon dioxide, and lactic acid, which latter was also known to be produced. He held that these two latter substances were given off into the blood, while the nitrogenous constituent was used again to reproduce the original compound by the assistance of energy derived from food arriving in the blood. Although we know now that the only essential part of the contractile process is the separation of lactic acid, and on the surface of certain structural constituents of the muscle cells, which do not actually share in the chemical decomposition process, it is clear that if we regard Hermann's myosin as a name for these structures, his view was an important step in the right direction, although the giving off of carbon dioxide was not correctly interpreted.

Hermann was the first to show that electrical changes are an accompaniment of the activity of many *secreting glands.* He used the "alteration theory" to interpret the phenomena.

The second main region of his work, which was his chief occupation at Königsberg, was concerned with the analysis of *voice and speech.* In this his mathematical skill was of much service. Use was made of photographic registration and a method of magnifying the impressions on phonograph records was worked out. It is impossible to describe this work in detail, but the main point was the view of the composition of vowel sounds to which it led. This view was that vowel sounds are not merely characterised by harmonics of tones produced in the larynx, but that sounds are added to these by blowing through the mouth cavity, specially adjusted for each vowel sound.

Many other branches of physiological research received valuable contributions from Hermann's laboratory. Heat production, respiration, and the blood pigment may be mentioned. To him, as it appears, should be given the credit of pointing out that the *digestive processes* in general are of the nature of hydrolytic decompositions, and that their object is to afford the simple materials to be used by each cell for the building up of its own special products.

Not the least of his services to physiology was the bringing out of the 'Handbuch der Physiologie' in 1879—1883. Several of the articles are by Hermann himself, and the work as a whole gives a most valuable account of what was known at the time. Indeed, much of it may still be read with profit.

Hermann's success in research was due in great part to his exceptional skill in the design, construction, and use of apparatus as needed for the problems on which he was engaged. Most of these problems depended for their solution on the accurate measurement of physical quantities.

Hermann was strongly of the opinion that physiology must be studied for its own sake, not merely for its use in the practice of medicine. He refers to the fundamental problems which are opened up when courage is found to attack the phenomena of life at their source in the "organisation of the elementary parts." That such knowledge, sooner or later, has its practical application is illustrated by the use to which Hermann's discoveries in the field of the electrical phenomena of muscle has been put in the methods of diagnosis by electrocardiographic records.

W. M. B.

SIR J. HUTCHINSON, 1828–1913.

Sir Jonathan Hutchinson was born at Selby on July 23, 1828. He was of Quaker parentage, and through his life was a member of that religious body. He received the beginnings of his medical education at the York Medical School and County Hospital, and he was apprenticed at the age of 16 in the old-fashioned way to a medical practitioner. At the York Medical School he came under the influence of an able though eccentric teacher, Dr. Laycock, afterwards Professor of Medicine at the University of Edinburgh. It was possibly from Laycock that Hutchinson received the impetus towards the study of heredity in the causation of disease and the importance of the observation of physiognomy in clinical medicine. His medical studies were further pursued at St. Bartholomew's Hospital under Sir James Paget, and he obtained his qualifying diploma in 1850. He thereupon commenced practice in the city of London, but his time and energy were rapidly absorbed by a succession of hospital appointments. The hospitals for diseases of the skin, of the eye, of the genito-urinary organs, of the lungs, secured either in turn or contemporaneously his ardent devotion, and it may be said that at each one he gained and added some new material for clinical medicine. At length he became attached to the staff of the London Hospital, where, as a general surgeon till 1883, his activities were mainly directed. But he continued his devotion to diseases of the eye and of the skin more or less during all this period, and his wide multiform experience was utilised in teaching, formally or informally, almost to the end of his life.

His most important contributions to medical science concerned syphilis, in which it may truly be said that his researches made a definite landmark in medical knowledge.

The morbid condition in children, which had previously been designated strumous corneitis, he proved to be an interstitial inflammation due to inherited syphilis. He showed, further, that this special form of inflammation was accompanied by a characteristic alteration of the permanent upper median incisor teeth, by a marked physiognomy, and often by a special form of deafness. His papers, recording many cases of this clinical group, appeared in the Ophthalmic Hospital reports, and were subsequently collected in a clinical memoir of very great value. Their conclusions have received complete verification and acceptance, and they mark the starting point of the later scientific studies on hereditary syphilis.

Hutchinson's article on "Constitutional Syphilis" in 'Reynolds's System of Medicine' was scarcely less remarkable by way of valuable generalisation than his elucidation of the symptomatology of the hereditary form of the disease. In this article he maintained that the proper place for syphilis in the classification of diseases was side by side with the acute infectious fevers. He claimed syphilis as a "thinned out" fever, with its incubation, its acute invasion, its efflorescence, its developed symptoms, and its sequelæ. The sequelæ had been previously designated as belonging to the tertiary stage of the disease. In all these stages the analogies and affinities with the acute specifics were pointed out.

Hutchinson made valuable clinical and pathological studies of dental defects in children who had been the subjects of convulsions in infancy, and likewise suffered from lamellar cataract of the lens.

He drew attention to several manifestations of gouty inheritance hitherto unnoticed, especially in regard to hæmorrhagic troubles, and he made an exhaustive investigation of the clinical history of tobacco amblyopia.

In his lectures on the "pedigree of disease," he collected and elucidated many varied and important facts bearing on idiosyncracies (*a*) in disease, (*b*) in the reaction in different individuals to various foods, (*c*) in the reaction in different individuals to certain drugs.

For many years Hutchinson made careful study of the natural history of leprosy, and, in the course of his enquiries on the geographical distribution of this malady, he visited Norway, South Africa, and India. Some of these journeys were made in advanced life. He maintained that leprosy was a food disease, and he marshalled a large body of arguments in support of his contention that the eating of decomposing fish was the essential factor. He accepted the later bacteriological findings of Hansen, but claimed that they could be grafted on to his own doctrine. He failed to secure the acceptance of his contention, and this is probably the solitary instance of his failure.

Hutchinson was a master of note taking. He took great pains in recording individual cases, laying much stress on the distribution of the lesions as to their symmetry or asymmetry, and he introduced a simple graphic "space for time" diagram, in which he allotted an equally wide column for any given number of months or years, and inserted the date when the important symptoms appeared during the evolution of the disease. Thus his life histories yielded interesting facts and generalisations as to the usual period of onset and the duration of given symptoms, and the frequency with which different groups of symptoms occurred.

He was a great collector of pathological specimens and of drawings of noteworthy skin conditions, etc., and he was an enthusiastic exponent of museums. He was one of the best clinical teachers of his time. He threw a flood of light on the commonest diseases, and had that faculty of the scientific use of the imagination which enabled him to show the relationship of one disease with another, however far they might superficially seem to be apart.

Although excellent as a teacher of undergraduates, it is in connection with post-graduate instruction that he will be chiefly remembered. To the Polyclinic, in Chenies Street, he devoted much time, effort, and money. He gave regular demonstrations to medical men there. With this institution his own clinical museum was ultimately incorporated, and he spared no pains in inducing other fellow-workers to join in this important educational effort.

Hutchinson presided in successive years over several of the London medical societies, and one of his most important services to his profession was as secretary and inspirer of the New Sydenham Society. He organised the translation and publication of many foreign books, lectures, and pamphlets, and the reprinting of not a few important English memoirs.

Hutchinson held in turn every office of the College of Surgeons, including the Hunterian Professorship and the Presidency. He was elected a Fellow of the Royal Society in 1882 on account of his contributions to scientific medicine, and he was knighted in 1908.

It seems right to add to this record of Hutchinson's activities a brief note of his recreations at his Hindhead home. He was greatly interested in any opportunities which presented themselves of studying animal pathology, but, most of all, in developing an educational village museum, which should set forth in a homely way, geological, biological, and historical knowledge, by specimens, diagrams, and other illustrations. Here, or on a grassy platform close by, he gave short colloquial lectures on Sunday afternoons to village audiences, choosing for his topics some scientific material, interspersed with brief discourses on biography, history, and economics. He founded an educational museum also at Selby, his native town, and there also he gave regular Sunday afternoon addresses.

He died in the fulness of years, only desiring that he might be remembered as a "forward-looking man." It would be difficult to parallel a life which, almost to the end, was so full of ripe and varied observation, of philosophic review, and of beneficent activities, superposed on the career of a busy and successful surgeon.

T. B.

Sir VICTOR HORSLEY, 1857-1916.

This delayed obituary of the late Sir Victor Horsley pertains especially to his scientific work, because already his family history, upbringing, character, and career have been so admirably and judicially dealt with in 'A Study of his Life and Work,' by Stephen Paget, 1919.

Victor Alexander Haden Horsley was born on April 14, 1857, at 1, High Row, Kensington. His mother was a daughter of Charles Haden, the surgeon; his father was John Callcott Horsley, R.A. Victor was educated at Cranbrook School, and in January, 1874, he matriculated at the University of London. In 1875, having passed his preliminary scientific examination, he entered as a medical student at University College. Early in his student days he showed those distinctive qualities of originality of thought, combined with a conception of utility, industry, and pertinacity of purpose which rapidly developed into a remarkably brilliant and unique career as a scientist and surgeon, terminated only too early by one of the saddest fatalities in the Great War.

In 1880, at a time when Listerism and Spontaneous Generation were the burning subjects of scientific discussion, Horsley was engaged with another student (the writer) for seven months in investigating the question as to "the Existence of Bacteria or their Antecedents in Healthy Tissues," the positive results of which were shown in a paper published in the 'Journal of Physiology,' 1882.

He passed the final examination for the Membership of the Royal College of Surgeons, 1880, and became house-surgeon to Mr. John Marshall, who recognised his great scientific abilities. In 1881, he took the M.B. and B.S. degrees of the University of London, and was awarded the gold medal in Surgery. It so happened that an unusual number of brilliant students who have since attained to the highest positions in medicine and surgery obtained these degrees at the same time.

From 1882 to 1884 Horsley held the appointment of surgical registrar and assistant professor of pathology. During this time he was engaged in investigating the patellar knee-jerk and the results of these observations were published in 'Brain,' October, 1883. He also wrote a report on "Septic Bacteria and their Physiological Relations" for the Local Government Board. It was, however, in 1884 with Prof. Schafer that he began his great work on 'The Functions of the Cerebral Cortex,' and very soon he was applying the knowledge and experience gained by his experiments upon animals, for in December, 1884, he read, at a meeting of the Physiological Society, notes on four cases of injury of the brain illustrating the position of the motor centres. Moreover, now, at the age of 27 he published several valuable papers on cerebral function and made an important addition to knowledge by the demonstration of the existence of *nervi nervorum*.

In this year, having become engaged to Miss Eldred Bramwell, Horsley had to decide the lines of his future career, and it is interesting to note that in a letter he announced his intention to practise as a surgeon on the grounds that: "the most solid work in Pathology has been done by men in practice, and at least one will have an idea as to what is more likely to be practically useful in the way of research." Only men like Horsley with the extraordinary energy and powers of concentration on the scientific aspects of practice could combine the two departments with such brilliant success.

From 1884 to 1890 Horsley acted as Professor Superintendent of the Brown Institution and this period was probably the most fruitful of his scientific career. Although this institution has had many distinguished superintendents since, it has never flourished to the same degree as when he was its spiritual fire. During these six years he attracted a company of researchers, but he himself studied (1) The Localisation of Function in the Brain and the Pathology of Epilepsy in Canine Chorea; (2) The Thyroid Gland with especial Reference to Myxœdema and Cretinism; (3) The Protective Treatment of Rabies.

His work on the localisation of the brain was carried on first with Sir Edward Sharpey-Schafer and later on with the late Dr. C. E. Beevor.

His work on the thyroid gland and rabies was all, or nearly all of it, carried out by himself at the Brown Institution. The scientific acumen, courage, strength of will and determination of the man was shown in a remarkable manner in his successful efforts to stamp out rabies in this country by the muzzling of all dogs. Fortunately, Mr. Walter Long was, at the time, President of the Local Government Board, and to these two men, who had the courage of their convictions, the nation owes a deep debt of gratitude for resisting the clamorous attempts made by the public to relax or abolish the Acts until it was deemed safe so to do.

It was a similar but more protracted and bitter struggle that took place later between Horsley and a section of the public and their leader, Miss Frances Power Cobbe, who were militating to obtain total abolition of vivisection. He was able to show that their arguments were based upon a *suppressio veri*, whereas his arguments in favour of vivisection were all the more effective on account of his own valuable researches upon living animals. He conclusively demonstrated not only the great benefit humanity had derived from experiments upon animals, but that the animals themselves would be relieved from suffering by the knowledge obtained from legalised vivisection.

The dawn of the function of eudocrine glands arose in England with the clinical recognition of the association of thyroid insufficiency with the condition termed myxœdema. Claude Bernard, in his 'Physiologie Opératoire," 1879 edition, published the year after his death, speaking of the thyroid and supra-renal glands says: "Yet we know absolutely nothing of the function of these organs." It is a satisfaction to British biological science to recognise that the functions of these two glands were first illuminated by the brilliant researches of Horsley on the thyroid, and by those of Oliver and Schafer on the supra-renal.

In 1882 to 1883, the Swiss surgeons, Kocher and Reverdin, published their results of operations upon the thyroid gland for goitre, which was followed by "Myxœdeme Opératoire." In November, 1883, a committee of the Clinical Society recognising "the hitherto undreamt of importance of the thyroid gland," asked Horsley to study it by the experimental method. This he did upon monkeys, and a year later he gave two lectures at the University of London, embodying his results, entitled—"The Thyroid Gland: its Relation to the Pathology of Myxœdema and Cretinism, to the Question of the Surgical Treatment of Goitre, and to the General Nutrition of the Body." This title is comprehensive and exhibits the characteristics of Horsley, for it shows that he had studied the subject as a physiologist, pathologist, and a surgeon.

To Schiff, however, belongs the credit of having first produced an experimental *Cachexia strumipriva* with tremors in dogs and rodents. Horsley, in a letter to Schafer, remarks: "Well, we did four monkeys last week, and one of them has the same tremors." It seems that Horsley did not recognise in his report *how* the myxœdema was produced, and it was not until 1890, when he published in the 'British Medical Journal,' "A Note on the Possible Means of Arresting the Progress of Myxœdema, *Cachexia strumipriva*, and Allied Diseases," that he admitted the importance of the successful transplantation experiments of Schiff and von Eiselberg—and he then proposed that, as soon as opportunity offered, to try transplanting a portion of the thyroid gland of a sheep. A short time after, the treatment of myxœdema by transplantation of the gland was replaced by administration of it by the mouth. It is a remarkable biological fact that this gland, the secretion of which was once poured through a duct into the alimentary canal, is effective when administered by the mouth, whereas other ductless glands, *e.g.* the supra-renal, are not. Whether it was chance, or the knowledge of this biological fact, which led Dr. George Murray to try this treatment in practice, it has turned out to be one of the greatest advances in medical therapeutics.

Although the scientific work already mentioned as accomplished by Horsley had been sufficient to lead to his election in 1886 to the Fellowship of the Royal Society, his pre-eminence as a pioneer in Cerebral Localisation and its practical application to brain surgery will stand for all time. From 1884 to 1891, he published in the 'Philosophical Transactions,' in conjunction with Schafer, Beevor, Semon, Walter Spencer, and Gotch, eight papers, 525 pages in all, with 33 plates. Mr. Paget thus admirably sums up this work: "First, there was the more general work with Schafer, then the more special work with Beevor—the study of localisation in one species of monkey; the study of localisation, not only at the level of the surface of the brain, but at the level of the nerve fibres passing from the surfaces of the brain towards the spinal cord, and the single study of an anthropoid brain." The latter, on account of its importance, requires some fuller exposition on my part.

In 1890 Horsley and Beevor discovered definitely localised areas in the orang brain. In certain points of anatomical detail, and more especially the excitability of the post-central convolution, combined with the existence of intermediate inexcitable zones, their results were modified by the subsequent researches of Grünbaum and Sherrington. But they do not in any way detract from the value of Horsley's pioneer research and its application to cerebral surgery in man. The fact must also not be forgotten that Horsley was the first to operate in 1887 for tumour of the spinal cord on a case of Sir Wm. Gowers, and to remove it with successful results.

To continue Paget's account of the scientific researches of Horsley: "Then the special work with Semon, the study of the localisation of the centres for the movements of the larynx." The work with Walter Spencer was concerned with the control of hæmorrhage from the middle cerebral artery by compression of the common carotid. That with Gotch was a long and important paper upon observations upon the electro-motive changes in the mammalian spinal cord following electrical stimulation of the cortex cerebri.

The two fundamental principles shown by these observations were:—

"(1) Electro-motive changes occurred at the cut surface of the lower dorsal spinal cord, that is, below the spinal centres for the upper limb and above the spinal centres for the lower limb, only when the cortical centres for the lower limb were stimulated, but not otherwise.

"(2) The electro-motive change so produced was first a persistent stage, next a rhythmical stage answering to the tonic and clonic stages of the convulsions, and the conclusion was arrived at that during a cortical epileptiform discharge the electro-motive changes in the cord are exactly parallel, as regards the character of their sequence, to the convulsion of the muscles as recorded by the graphic method."

In 1886 he was appointed surgeon to the National Hospital for Epilepsy and Nervous Diseases—thus early in his career he obtained the opportunity he desired of applying his scientific knowledge and experience obtained by experiments upon animals to the surgery of the brain and spinal cord.

Horsley's skill as a surgeon in this speciality soon became unrivalled, and his fame spread abroad. It was not, however, until 1900 that he was appointed full surgeon at University College Hospital. Two years later he received the honour of knighthood. His practice now became very extensive, but he still found time to deliver many scientific addresses and lectures; and even as late as 1910 he devoted a considerable amount of time to experimental investigations, principally in conjunction with R. H. Clarke, on "The Intrinsic Fibres of the Cerebellum, its Nuclei and Efferent Tracts"; they also established the fact that the cerebellar cortex is practically inexcitable.

Sir Victor gave up his appointment at University College Hospital and his Professorship of Surgery at University College in 1906. About this period he commenced to turn his attention to medical politics and social questions, especially those of female suffrage and temperance, of which he became the great protagonist. Had he lived he would have seen many of the reforms he was pressing already adopted. This only shows how inestimable a benefit to others it is that some men should think differently and act differently to accepted customs and traditions.

In 1909 Sir Victor delivered the Linacre Lecture upon "The Function of the so-called Motor Area of the Brain." This is generally considered the most philosophical of all Horsley's writings, because it is enriched by twenty-three years' unrivalled experience of a physiologist, pathologist, and surgeon, and by the description of a case upon which he had operated and was able to show, as a result of the operation, that the Gyrus pre-centralis in man is the seat of representation of: (1) slight tactility, (2) topognosis, (3) muscular sense, (4) arthric sense, (5) stereognosis, (6) pain, and (7) movement.

Some of the scientific honours bestowed upon Sir Victor Horsley were:—The Royal Medal, 1894; M. D. Halle, 1894; Foreign Associate of the French Academy of Medicine, 1910; Member of the Royal Prussian Academy of Medicine, 1910; the Lannelongue Prize and Gold Medal, 1911; Member of Royal Society of Upsala, in succession to Lord Lister, 1912.

His publications, individual and combined, number 129, of which quite 30 are of outstanding importance to science.

Sir Victor's intention was to enter Parliament where, owing to his courage, indomitable energy, and medical knowledge and experience in its widest sense, he would have been of immense help to the Ministry of Health. But it was ordained otherwise—he went on another crusade, as surgeon in the R.A.M.C., first in Egypt then in Mesopotamia—the stirring work he did in the army is well recorded in his letters home, and his death from heat-stroke at the age of 59 (July, 1916), unfortunately ended the career of one of the greatest surgeons and scientists England has produced—his loss irreparable to those who, like myself, had the privilege of a long and unbroken friendship—and we hope that now his numerous friends and admirers will help us in the founding of a memorial lectureship bearing his name.

F. W. M.

OCTAVIUS PICKARD-CAMBRIDGE, 1828–1917.

The Rev. Octavius Pickard-Cambridge, who died on March 9, 1917, was the fifth son of the Rector and Squire of Bloxworth, Dorset, the Rev. George Pickard, who added the name of Cambridge on succeeding to the property of his cousin, C. O. Cambridge. He was born November 3, 1828, at the Rectory, where, as Rector of Bloxworth, he lived for nearly fifty years.

Pickard-Cambridge did not go to a Public School, but was for two years, in the middle forties, a pupil of the Rev. William Barnes, the famous Dorset poet, who was then keeping a school in Dorchester. To this inspiring teacher he owed the foundation of the literary power which is of inestimable value in the presentation of scientific work.

"It was appropriate that his most finished piece of writing should have been the memoir of his old teacher and friend, which he contributed to the 'Proceedings' of the Dorset Field Club in 1887—a warm tribute of admiration and gratitude, together with an appreciation of Mr. Barnes's poems, which, while disowning all attempt at criticism, shows real critical power and insight into the nature of poetry."*

Mr. A. W. Pickard-Cambridge well remembers, too, how his father's help in holiday tasks made the literary subjects living and intelligible.

While working with Mr. Barnes he learned the violin, confirming and strengthening the natural gift for music, which meant so much for himself, as well as others, throughout his life. His skill in drawing, almost essential for the work of a naturalist, must have been apparent at an early age, for his sketch-book of 1852–3 "contains some exquisite pencil-sketches, chiefly of the churches of the country round Hatch Beauchamp."

Apart from his work with Mr. Barnes, Pickard-Cambridge enjoyed the pursuits of the country—gardening, bee-keeping, and, above all, shooting. In 1849 he went to London to enter on a two years' course of study for the Bar, but, although the experience was valuable, the work and the life did not suit him, and were not continued. Then, for the two years 1852–3, he read with a tutor at Hatch Beauchamp, Somerset, and, in 1855, entered University College, Durham, to prepare for Holy Orders and to take his degree—B.A. in 1858, M.A. in 1859. At College he worked hard, and entered fully and with spirit into the many-sided life of the University.

In 1858 he was ordained, and held the curacy of Scarisbrick, in the diocese of Chester, with a stipend of £60 a year. He was at Scarisbrick when the "Origin of Species" appeared in 1859, and remembered with amusement the denunciations of Darwin by the local clergy.

In 1860, Pickard-Cambridge left Scarisbrick, to help, and in 1868 to succeed, his father, as Rector of Bloxworth and Winterbourne Tomson.

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* This and all succeeding quotations without reference are from the 'Memoir of the Reverend Octavius Pickard-Cambridge,' by his son, A. W. Pickard-Cambridge, Fellow of Balliol College, Oxford, printed for private circulation, 1918.

The latter, a mere handful of people, often less than twenty in all, living round a little church two miles away, was the cause of much wasted energy, for there were two other churches near at hand. Relief came about 1890, when the church was closed. In the meantime, "for about thirty years, as Curate or Rector, he had walked over Sunday after Sunday in all weathers, sometimes returning drenched to the skin, sometimes with his beard bristling with long icicles, sometimes almost 'done up' with the mid-day heat. He calculated that he had covered about 7000 miles in coming and going between Bloxworth and Tomson."

As curate to his father, he lived in "The Cottage" near the Rectory at Bloxworth. On April 19, 1866, he married Miss Rose Wallace, the daughter of the Rev. James Lloyd Wallace, Head Master of the Grammar School at Sevenoaks. They met two years before, on his first visit to the Continent. Until her death in August, 1910, she gave him unwearied and devoted help in the varied duties of the parish and home. They had six sons, of whom the second died in infancy. "Not one of us," writes A. W. Pickard-Cambridge, "would hesitate to attribute any success we may have achieved in different ways, in the first place, to her patient and thorough teaching." And of his father—"To his family, my father was always a boy among boys; he shared all our pursuits and amusements, and, without knowing how much we were gaining, we acquired from him a delight in nature and a habit of observing natural objects, which has been one of the best things in our lives. He would take any pains for our pleasure. . . . He rarely went out for a walk or for a collecting expedition without one or more of us, and we had no greater pleasure than his companionship, for he was always fresh and never seemed to grow old. . . . When, as we grew older, we came to hold views different from his own on many matters, it made no difference to the happiness of our companionship with him."

His son writes of the Bloxworth life: "From the time of his entry into the Rectory until his death, my father lived the uneventful life of a country parson, seldom leaving home, except for a few days' collecting from time to time, or a meeting of the Dorset Field Club, or a brief visit to London or Oxford, principally for work in natural history museums, or sometimes to spend a few days with a brother naturalist. The contents of his diaries show some of the interests which entered into a singularly happy and contented life—the dates of the planting and digging of potatoes—of the first rhubarb, asparagus, peas, or strawberries—of the 'meets' of foxhounds at Bloxworth or the 'Red Post'—of the buying and selling of pigs—of concerts in his own or neighbouring villages. . . . He was an old-fashioned High Churchman, and took a somewhat severe view of Dissent—though no Dissenter ever found him lacking in charity in times of need. . . . He thoroughly understood his parishioners, nearly all of whom were farm labourers of the type which prevails in the West of England; he knew their work and their life and its conditions as well as they did; and to every one in the parish he was always ready to give advice and help on any matter on which help was needed; he had, and retained to his death, their trust and affection. . . . They came to him naturally in any trouble or difficulty; he told them faithfully when he thought they were wrong, and did not always wait to be asked his opinion. . . . When one looks back over his life as Rector for nearly fifty years, and the longer life, which was almost all passed in Bloxworth, it is no wonder that one of the older farm labourers—a man not much given to expressing emotion—should have said, when my father passed away, 'There, 'tis the end of all things to we.' "

During the period of his curacy at Bloxworth, Pickard-Cambridge went abroad twice with a pupil, Mr. O. Bradshaw, spending nearly the whole of 1864 in various parts of the Continent, in Egypt, and Corfu. On January 10, 1865, they started again, and, after nearly three months in Italy, sailed to Alexandria and Jaffa for a two-months' tour in Palestine and Syria. Then Greece, Austria, Germany, and home at the end of the year by way of Holland and Belgium.

There can be no doubt that the experiences of these two years were of the utmost value to one who was to spend the rest of his long life in a somewhat remote country parish. "They were something more than a passing phase in his life. He never forgot the fascination of foreign scenery and architecture, the delight and freshness of collecting exotic creatures, and the manifold experiences of his travels, especially in Egypt and Syria. He used to speak of these with a pleasure which seemed always alive, and his travels gave a reality and interest to his life-long correspondence with foreign naturalists and his continual work at exotic collections sent to him by them and others." His diary, begun when he was 21, and kept up until just before his death, gives an account of his travels, from which many passages of great interest, often brightened by flashes of an incisive humour, are reprinted by his son.

From the above brief account of his life and surroundings, it is clear that Pickard-Cambridge had every chance of developing his strong innate love of Natural History. Before he was seven he had captured *Colias hyale*, a butterfly rarely seen in this country, and, in doing so, had probably received his earliest impulse. His first published observation, on an almost white Willow Wren, appeared in the 'Zoologist' for 1852. Two years later, accompanied by the great Entomologist, Frederick Bond, he paid the first of many visits to the New Forest. It was probably in this year, 1854, that he first entered upon the scientific work of his life, his interest being stimulated by the earlier writings of John Blackwall, the leading authority on British spiders. These publications were first brought to his notice by an Entomological friend, R. H. Meade. He soon entered into correspondence with Blackwall, and, in 1860, visited him at Llanrwst, where they had long and frequent discussions over the 'Origin,' which had recently appeared.

Nineteen years later I made the same pilgrimage, and well remember the stimulating enthusiasm of the aged naturalist. Discussing with considerable scepticism the belief that the bite of certain spiders was poisonous to man, Blackwall told me that he had often received monstrous tropical species with the most sinister reputation, and I clearly recall his pathetic tone of disappointment as he said :—

"They might have had cheek or lip, but not one of them would bite me!"

Had not my interests been already fixed, here was enthusiasm at the age of 89, which might well have persuaded me to be an arachnologist.

Except for two notes on "Abstinence of a Spider," in the 'Zoologist' for 1852 and 1853, Pickard-Cambridge did not publish anything upon the Arachnida until 1859, when he brought out "Remarks on Arachnida, taken chiefly in Dorsetshire and Hampshire" ('Zoologist,' p. 6493). From this time, until within three years of his death, he published papers on the subject nearly every year—often several in a year. He attained the highest rank as an arachnologist with such rapidity that Blackwall was glad to avail himself of his help in the publication, between 1861 and 1864, of his greatest work, 'British and Irish Spiders' (Ray Society). Pickard-Cambridge's principal monograph was the volume on Arachnida in the "Biologia Centrali-Americana," a very serious undertaking, occupying much of his time between 1883 and 1902. In this, and some of his other works, he was assisted by his nephew, F. O. Pickard-Cambridge, a very able naturalist too early lost to science. Of even greater importance in helping on the subject were his series of standard works on the British Arachnida—"Spiders of Dorset" (Pt. I, 1879; Pt. II, 1881), "British Species of Phalangidea' (1890), "British Species of Chernetidea" (1892), "List of British and Irish Spiders" (1900), and many shorter papers, bringing the first-named up to date. In fact, the greater part of his work appeared in the form of a succession of papers in the 'Zoologist,' 'Annals and Magazine of Natural History,' and publications of the Linnean and Zoological Socities, and the Dorset Natural History and Antiquarian Field Club. A complete list of his publications occupies seventeen pages of his son's memoir, and of these, eleven deal with the Arachnida.

He was elected F.R.S. in 1887.

His collection of Arachnida, and equally splendid library, rich in manuscript notes, he bequeathed to the University of Oxford. The specimens, which include the Blackwall series, now rest in the Hope Department, upon the shelves prepared for them in the "Den" at Bloxworth in 1884.

With all his activity in Arachnology, Pickard-Cambridge always retained a keen interest in other branches of Natural History, as the list of his recorded observations, especially upon birds and Lepidoptera, abundantly proves. Furthermore, his note-books contain a store of observations on birds, from which it is hoped that a selection may be published at some future time.

It has already been said that he was from the very first in sympathy with the views of Charles Darwin on the origin of species by Natural Selection. He differed from Darwin, and agreed with Wallace's later opinions on the theory of Sexual Selection, believing, as he wrote in 1869, that there is "something in the male organisation of a special, and sexual nature, which, of its own vital force, develops the remarkable male peculiarities so commonly seen, and of no imaginable use to that sex. In as far as these peculiarities show a great vital power, they point out to us the finest and strongest individuals of the sex, and show us which of them would most certainly appropriate to themselves the best and greatest number of females, and leave behind them the strongest and greatest number of progeny. And here would come in, as it appears to me, the proper application of Darwin's theory of Natural Selection; for the possessors of greatest vital power being those most frequently produced and reproduced, the external signs of it would go on developing in an ever-increasing exaggeration, only to be checked where it became really detrimental in some respect or other to the individual."* This is essentially the same as Wallace's view, arrived at much earlier than Wallace. It encounters one main difficulty—that there is no valid reason why one colour should need more vital power for its production than another, an objection especially obvious in structural colours dependent upon the precise thinness of plates or intervals between striæ, etc.

In writing this notice, I owe almost everything to the memoir printed in 1918 for private circulation by Mr. A. W. Pickard-Cambridge, who has entered with the most complete sympathy and insight into every side of his father's life. A copy, presented by the author, is in the library of the Royal Society.

E. B. P.

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* From a letter written to A. R. Wallace in 1869 and quoted in "Darwinism," 1889, p. 296, n. 1.

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